## STUDIES ON ALLELOPATHIC POTENTIALS OF SOME RANGE GRASS SPECIES IN BUNDELKHAND REGION

THESIS SUBMITTED
TO THE BUNDELKHAND UNIVERSITY, JHANSI
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN BOTANY

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#### SUPERVISOR'S CERTIFICATE

This is to certify that this work entitled "Studies on allelopathic potentials of some range grass species in Bundelkhand region" is an original piece of research work done by Sadhana Rai, M.Sc. (Botany) under my guidance and supervision for the degree of Doctor of Philosophy in Botany of Bundelkhand University, Jhansi (UP), India.

#### I further certify that:-

- (i) the thesis has been duly completed,
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- the candidate has worked under me for more than 24 months at the Institute (iii) from the date of registration,
- (iv) the thesis fulfils the requirements of the ordinance relating to the Ph.D. degree of the University, and
- (v) it is up to the standard both in respect of the contents and literary presentation for being referred to examiners.

(DR. U.N. SINGH)

#### **DECLARATION**

I hereby declared that the thesis, entitled "Studies on allelopathic potentials of some range grass species in Bundelkhand region" being submitted for the Degree of Doctor of Philosophy in Botany of the Bundelkhand University, Jhansi (U.P.) is an original piece of research work done by me and to the best of my knowledge and belief, is not substantially the same as one which has already been submitted for the degree or any other academic qualification at any other University or examining body in India or in any other country.

Dated: 14/12/98

Ledhara Rai (SADHANA RAI)

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Scathana Roi

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## **CONTENTS**

SI.No.	Chapter	Page No.
01	Introduction	1-18
02	Review of Literature	19-46
03	Material and Methods	47-57
04	Results	58-91
05	Discussion	92-117
06	Summary	118-122
07	Bibliography	I-XXI
08	Appendices	I-VII

## LIST OF TABLES

Table No.	Title
1	Sprays used for detecting phenolic compounds on paper chromatogram (PC)
2	Solvent used for paper chromatographic analysis of phenolics
3	Test weight of range grasses (Weight of 1000 seeds)
4	Germination (%) of freshly collected spikelets and seeds
5a	Effect of scarification and chemical treatments on germination (%) of
	spikelets (freshly collected)
5b	Effect of scarification and chemical treatments on germination (%) of
	spikelets (at nine months storage)
6a	Effect of storage on spikelets and its germination (%)
6b	Effect of storage of spikelets and removal of glumes (seeds) on germination
7	Rate of germination of spikelets and seeds of range grasses
8	Rf. values of cyanidin glycosides (anthocyanin) and cyanidin (anthocyanidin)
	extracted from the diaspore of P. pedicellatum
9	Rf. values of cyanidin glycosides (anthocyanin) and cyanidin (anthocyanidin)
	extracted from the diaspore of <i>B. intermedia</i>
10	Rf. values, U.V. absorption and colour reactions of phenolics isolated from
	the diaspore of <i>C. fulvus</i>
11	Rf. values, U.V. absorption and colour reactions of phenolics isolated from
	the diaspore of <i>P. pedicellatum</i>
12	Rf. values, U.V. absorption and colour reactions of phenolics isolated from
	the diaspore of <i>D. annulatum</i>
13	Rf. values, U.V. absorption and colour reactions of phenolics isolated from
	the diaspore of <i>B. intermedia</i>
14	Rf. values, U.V. absorption and colour reactions of phenolics isolated from
	the diaspore of <i>P. maximum</i>
15a	Effect of C. fulvus diaspore extract on germination and growth of root and
	shoot of R. sativus

15b	Effect of C. fulvus diaspore extract on germination and growth of root and shoot of
100	V. radiatus
15c	Effect of C. fulvus diaspore extract on germination and growth of root and shoot of
	C. fulvus
16a	Effect of P. pedicellatum diaspore extract on germination and growth of root and
	shoot of R. sativus
16b	Effect of P. pedicellatum diaspore extract on germination and growth of root and
	shoot of <i>V. radiatus</i>
16c	Effect of P. pedicellatum diaspore extract on germination and growth of root and
	shoot of <i>P. pedicellatum</i>
17a	Effect of D. annulatum diaspore extract on germination and growth of root and
	shoot of <i>R. sativus</i>
17b	Effect of D. annulatum diaspore extract on germination and growth of root and
	shoot of V. radiatus
17c	Effect of D. annulatum diaspore extract on germination and growth of root and
	shoot of <i>D. annulatum</i>
18a	Effect of B. intermedia diaspore extract on germination and growth of root and shoot
	of <i>R. sativus</i>
18b	Effect of B. intermedia diaspore extract on germination and growth of root and shoot
	of <i>V. radiatus</i>
18c	Effect of B. intermedia diaspore extract on germination and growth of root and shoot
	of B. intermedia
19a	Effect of P. maximum diaspore extract on germination and growth of root and shoot
	of <i>R. sativus</i>
19b	Effect of P. maximum diaspore extract on germination and growth of root and shoot
	of V. radiatus
19c	Effect of P. maximum diaspore extract on germination and growth of root and shoot
	of <i>P. maximum</i>
20	Phenolic compounds encountered in the dispersal units of range grasses

## **LIST OF FIGURES**

Fig No.	Title
1	Paper chromatography methodology
2	Effect of scarification and chemical treatments on germination (%) of freshl collected (A) and nine month of storage (B) of spikelets of range grasses
3	Effect of storage of spikelets (A) and removal of glumes (seeds, B) of germination percentage of range grasses
4	Rate of germination of spikelets and seeds of range grasses (A, B, C, D an E)
5	Effect of <i>C. fulvus</i> diaspore extract on germination and growth of root an shoot of <i>R. sativus</i> (A), <i>V. radiatus</i> (B) and <i>C. fulvus</i> (C)
3	Effect of <i>P. pedicellatum</i> diaspore extract on germination and growth of rocand shoot of <i>R. sativus</i> (A), <i>V. radiatus</i> (B) and <i>P. pedicellatum</i> (C)
7	Effect of <i>D. annulatum</i> diaspore extract on germination and growth of rocand shoot of <i>R. sativus</i> (A), <i>V. radiatus</i> (B) and <i>D. annulatum</i> (C)
3	Effect of <i>B. intermedia</i> diaspore extract on germination and growth of rocand shoot of <i>R. sativus</i> (A), <i>V. radiatus</i> (B) and <i>B. intermedia</i> (C)
9	Effect of <i>P. maximum</i> diaspore extract on germination and growth of root and shoot of <i>R. sativus</i> (A), <i>V. radiatus</i> (B) and <i>P. maximum</i> (C)
10	Phenolic and hydroxy cinnamic acids encountered in the dispersal units or range grasses
11	Flavonoids (aglycone) encountered in the dispersal units of range grasses

## LIST OF PLATES

Plate	Title	
No.		
1 A	stand of <i>B. intermedia</i>	
<u>2</u> A	stand of <i>C. fulvus</i>	
3 A	stand of <i>D. annulatum</i>	
4 A	stand of <i>P. maximum</i>	
5 A	stand of <i>P. pedicellatum</i>	
3 D	sispersal units (Spikelets) of five range grasses	
7 A	chromatography chamber	
3 A	queous extracts of diaspore of five range grasses	
	Chromatogram in BAW (n-butanol - acetic acid - water (4:1:5, upper laye nd 15% AA (15% acetic acid)	
	ffect of <i>C. fulvus</i> diaspore extract on germination and growth of root an hoot of <i>V. radiatus</i>	
	ffect of <i>P. pedicellatum</i> diaspore extract on germination and growth of roon and shoot of <i>V. radiatus</i>	
	ffect of <i>D. annulatum</i> diaspore extract on germination and growth of roon and shoot of <i>V. radiatus</i>	
	ffect of <i>B. intermedia</i> diaspore extract on germination and growth of roon and shoot of <i>V. radiatus</i>	
	ffect of <i>P. maximum</i> diaspore extract on germination and growth of root an hoot of <i>V. radiatus</i>	

## LIST OF APPENDICES

Appendix		Title	
No.			
1	Ana	alysis of variance of test weight of range grasses	
11		alysis of variance of scarification and chemical treatment on mination of spikelets at one month	
111		alysis of variance of scarification and chemical treatment on mination of spikelets at nine month	
IV	Ana	alysis of variance of storage on germination	
Va		alysis of variance of leachate of <i>C. fulvus</i> on germination, root and ot length of <i>R. sativus</i>	
Vb		alysis of variance of leachate of <i>C. fulvus</i> on germination, root and ot length of <i>V. radiatus</i>	
Vc		alysis of variance of leachate of <i>C. fulvus</i> on germination, root and ot length of <i>C. fulvus</i>	
Vla		alysis of variance of leachate of <i>P. pedicellatum</i> on germination, root shoot length of <i>R. sativus</i>	
VIb		alysis of variance of leachate of <i>P. pedicellatum</i> on germination, root shoot length of <i>V. radiatus</i>	
VIc		alysis of variance of leachate of <i>P. pedicellatum</i> on germination, root shoot length of <i>P. pedicellatum</i>	
VIIa		alysis of variance of leachate of <i>D. annulatum</i> on germination, root and ot length of <i>R. sativus</i>	

VIIb	Analysis of variance of leachate of <i>D. annulatum</i> on germination, root and shoot length of <i>V. radiatus</i>
VIIc	Analysis of variance of leachate of <i>D. annulatum</i> on germination, root and shoot length of <i>D. annulatum</i>
VIIIa	Analysis of variance of leachate of <i>B. intermedia</i> on germination, root and shoot length of <i>R. sativus</i>
VIIIb	Analysis of variance of leachate of $\it B.~intermedia$ on germination, root and shoot length of $\it V.~radiatus$
VIIIc	Analysis of variance of leachate of <i>B. intermedia</i> on germination, root and shoot length of <i>B. intermedia</i>
IXa	Analysis of variance of leachate of <i>P. maximum</i> on germination, root and shoot length of <i>R. sativus</i>
IXb	Analysis of variance of leachate of <i>P. maximum</i> on germination, root and shoot length of <i>V. radiatus</i>
IXc	Analysis of variance of leachate of <i>P. maximum</i> on germination, root and shoot length of <i>P. maximum</i>

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# INTRODUCTION

## INTRODUCTION

The grass cultivated area devoted to fodder production in India is disproportionate and due to remote possibility of any significant increase in the acreage owing to priority for human food production, the livestock industry substantially depends on the natural grassland constituted by some range grasses including some indigenous species in particulars. Now, it has been well recognised that due to the past over use, continuous and close grazing as well as lack of scientific management of these vast national resources had deteriorated the vital economy of the country considerably. Although, the improvement and management of grasslands on scientific basis to maintain these natural resources in the highest of productivity had attracted the national attention in the early fifties of this country but the state of knowledge and the research in this field has not yet reached a stage at which a good overall picture can be drawn. Some amount of work on seed production, germination etc. has been carried out in Indian Grassland and Fodder Research Institute, Jhansi and elsewhere. A little is known about the presence of germination inhibitors, retention or loss of viability during storage and many other related ecophysiological aspects, which are of basic values to understand the germination of perennial grass species as reproduction from seed is very common in perennial grasslands (Lieth, 1960).

Inhibition of seed germination a commonly known allelopathic phenomenon is of wide occurrence in plant Kingdom (Evenari, 1961; del Moral and Cates, 1971; Lodhi, 1976;). Presence of germination inhibitors in the seeds of many desert plants is important

for their survival. It is a kind of germination control mechanisms, which help the plants to eliminate competition. By preventing germination under established plants, sufficient space as well as more moisture per plant is assured.

In poaceae (Gramineae), the dispersal unit (which is spikelet or a group of spikelets) consists of caryopsis enclosed in glumes, lemmas, paleas etc. Removal of caryopsis from the enclosing glun es etc. (husk) has been reported to have an enhanced effect on percentage germination of seeds. Many workers viz., Alkarnine, 1944; Elliot and Leopold, 1953; Lahiri and Kharbanda, 1962; Mott, 1974; Martin, 1975; Hagon, 1976; Tothill, 1977; Pandeya and Jayar, 1978; Parihar etcal. 1984 a, b and many other have shown that seeds of various grasses germinate better when the glumes are removed.

Inhibition of germination by glumes extract/spikelet leachate of the grass seed (caryopsis) as well as of test species have been observed and identification of inhibitors have been done in a number of cases.

Most of the chemical inhibitors are compounds termed as 'secondary substances'. Now, there are plenty of evidences indicating the secondary compounds in seed act as preservatives i.e. prevent the seeds from decaying (Swain, 1979; Rice, 1984; Tang and Zang, 1986) as most of the seeds that do not germinate rapidly after landing in the soil.

It is also evident that secondary compounds in seeds also act as a feeding deterrent for a variety of phy ophagous insects and thus constitute the chemical defense against herbivore predators which becomes essential during the course of evolution of a particular community (Janzer, 1977, 83; Seigler, 1977).

On the basis of a work carried out on germination inhibitors Kefeli and Dashak (1984) stated "Unfortunately these chemicals are still considered by many as inhibitors".

The study of seed germination emphasizing germination inhibitors present in the seeds are of practical importance for the formulation of proper management plans for the grazing lands in India and elsewhere. Therefore, studies on "allelopathic potentials of some range grass species belonging to <u>Sehima-Dichanthium</u> grass cover of India (Dabadghao and Shankarnarayan, 1973) were selected for the present study. The work has been carried out in the Research Laboratory of the Department of Botany, D.V. Post-Graduate College, Orai and Indian Grassland and Fodder Research Institute, Jhansi with the following objectives:

- 1. To assess the seed dormancy, germinability and viability including effects of scarification/chemical treatments on dormancy and seed germinability.
- 2. To obtain information on the effect of storage (seed age) and removal of glumes on germination so as to know the role of enclosing glumes etc. on seed (caryopsis) germinability and viability.
- To isolate and characterize the germination inhibitors present in the dispersal unit by the combined use of chromatography, absorption spectroscopy and colour reaction.
- 4. To conduct bioassay studies on the compounds extracted from the dispersal unit and using these compounds on the respective grass seed (caryopsis) and two test species.
- 5. To explain the possible ecological significance of these chemical compounds present in the dispersal unit in the light of recent work carried out on this important field of allelopathy.

## Ecological characteristics of grasses studied

Bothriochloa intermedia (R.Br.), A. camus ; Andropogon intermedius R. Br.

Common names: Forest blue grass (Australia), lautoka grass (Feji), Australian blue-stem (United States).

Natural habitat: Open forest on heavier soils.

Distribution: Africa, India to Australia and the Pacific, introduced to united states.

Description: An erect to geniculately ascending branched perennial bunch grass (plate 1) to about 1 m height, the culms, often rooting at the lower nodes or, rarely, producing stolon bearing pale green to purplish raceme, simple or occasionally divided, arranged fairly densely about a central axis, the sessile pitted with a single tiny hole like a pinprick. Blade linear lanceolate and tapering gradually from the base to a fine hair like point. Ligule inconspicuous, short membranous and backed by sparse white hairs. Sheath glabrous and slightly compressed. Culms round, slender, glabrous. Roots coarse, aromatic.

Season of growth: Summer and rainy.

Frost tolerance: It survives seasonal frosts though the culms may be frosted. It is not tolerant as <u>D</u>. sericeum.

**Drought tolerance :** In Queensland, and Australia, it occurs in the 700-800 mm rainfall belt. It is fairly tolerant of drought conditions.

Tolerance to flooding: It will tolerate short term flooding.

**Soil requirements:** It occurs mainly on heavy clay loam to clay soils often derived from basalt in Queensland, and on heavier alluvial soils.

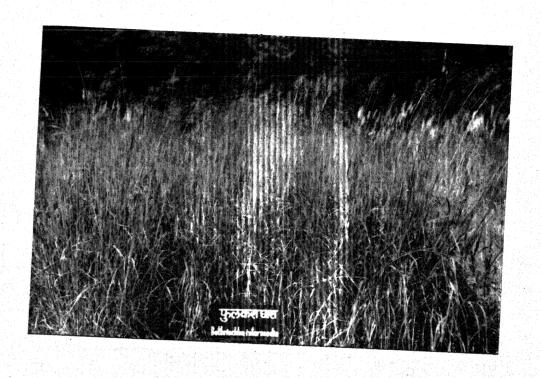


Plate -1. A STAND OF BOTHRIOCHLOA INTERMEDIA

.Tolerance to salinity: Moderate.

Fertilizer requirements: It is not usually fertilized but will respond to nitrogen.

Ability to spread naturally: It will spread slowly by seed.

Land preparation for establishment: The land can be fully prepared for seedlings or roughly prepared for rooted slips.

Sowing methods: It is propagated by rooted slip in India.

Response to defoliation: It will stand heavy grazing up to one beast to 0.4 ha at Gayndah, Queensland (700 mm rainfall with summer dominance, Skerman and Riveros, 1990).

Grazing management: Under experimental conditions at Gayndah, Queensland, a stocking system of two weeks grazing, six weeks rest was adopted. In this environment it was shown that utilizing the native pasture (e.g. <u>Bothriochloa intermedia</u>, <u>Heteropogon contortus</u> dominant) for summer and autumn grazing and sown pasture (e.g. green panic and lucern) for winter and early summer grazing improved annual production without sown pasture, winter/spring grazing of 0.74, 1.24 or 2.47 animals per hectare increased the basal cover of <u>B</u>. <u>intermedia</u> and <u>H</u>. <u>contortus</u> at the expense of other species and improved the sward. Continuous summer grazing reduced the percentage of <u>B</u>. <u>intermedia</u> at the expense of <u>Dichanthium</u> spp. Rotational grazing resulted in an increase in <u>B</u>. <u>intermedia</u>. Genetics and reproduction: 2 n = 40, 50,60,80.

Suitability for hay and silage: It makes quite useful hay in Australia, the United Republic of Tanzania and India. In the Kangara district in Punjab, <u>B. intermedia</u> top dressed with 28 kgN/ha in June and harvested in early September gave the highest yields and the best

quality hay (Narayanan and Dabadghao, 1972).

Palatability: It is a palatable grass in Queensland and India. In Ghana it is regarded as unacceptable to livestock. At Richmond, New Southwales, dairy cattle refused to eat it both in the young stage and later. It is some what aromatic.

Seed production and harvesting: In Queensland it seeds from mid summer (December) to late autumn. No seed is harvested commercially. In India, mature seeds are harvested during October-November.

Value for erosion control: It is useful, but there are better grasses, e.g. <u>B</u>. <u>insculpta</u>, suited to similar soils.

Diseases It is often attacked by smut.

**Economics**: It is very useful native grass for beef cattle in central and southern coastal queensland, Australia.

Main attributes: A palatable native grass which will tolerate drought condition and survive annual burning. It utilizes heavy soils well.

Main deficiencies: In Ghana it is unpalatable. It also invades lawns in the coastal savannahs.

## Chrysopogon fulvus (Spreng) Chiov.

Common names: Dhawlu grass (India).

Natural habitat: It grows on rocky gravelly red soil.

Distribution: Burma, Cyelon, India, Pakistan especially in hilly area upto 1830 m.

**Description:** Perennial herbs: Culms tufted, 30-180 cm tall (plate 2), erect or ascending, simple or sparingly branched, leafy towards the base, slender to stout, nodes glabrous.

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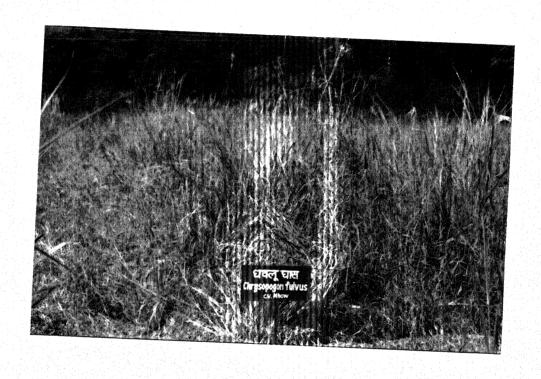


PLATE 2: A STAND OF CHRYSOPOGON FULVUS

Leaves: sheaths compressed, 6-20 cm long liquid a ciliated ring, blades flat, linear 7-28x0.4-0.9 cm, rigid, glabrous or sparsely hairy towards base, margins scaberulous, apex acute. Panicles: oblong-ovate to pyramidal 5-15 cm long, yellowish or purplish; rachis glabrous, scaberulous. Raceme: 1- nodded, branched whorled, 3-7 cm long, erect or spreading, simple, flexious, capillary with terminal traid of golden-brown spikelet of which 1-sessile and 2-pedicellated, callus bearded with fulvus hairs. Sessile spikelets 7.5-8 mm long, compressed, awned. Lower glumes chartaceous, laterally compressed, about 7.4 mm long 5-7 nerved, scabrid on nerves, 1- keeled; keel hairy towards acute to short aristulate apex. Upper glume chartaceous, boat shaped, 6.9-7.2 mm long, 3- nerved, 1keeled; keel hairy towards acute to short aristulate apex. The lower 1/3 of the keel hispid with fulvus hairy, apically produced into 13-14 mm long, arista; apex 2- toothed. Lower lemma empty, hyaline, linear, 6.3-7.0 mm long, nerveless, acute, epaleate upper lemma reduced to a hyaline base of geniculate awn, 2.5-3.5 cm long. Palea hyaline, linear, 3-3.5 mm long, Lodicules: 2. cuneate. Stamen 3: 3 anthers 3.5-4.5 mm long. Grain linear. Pedicel of peidcelled spikelets 7-8 mm long (excluding the arista). chartaceous, elliptic-lanceolate, 6.8-7.8 mm long, 7-nerved, narrowly 2-keeled; keels sparsely hairy, margins narrowly inflexed; apex acute or produced into 2-7 mm long arista. Upper glume similar, 3-nerved; margins ciliated, apex acute. Lower lemma empty, hyaline, lanceolate, 6.5-7 mm long, nerveless, acute, paleate or not. Upper lemma hyaline, lanceolate, 4.8-5 mm long, sub-acute at apex, paleate. Lodicules 2, cuneate. Stamens 3, anthers 4-4.5 mm long.

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Soil requirement: It is grown in rocky, gravelly red soils where soil depth is shallow.

Drought resistance: It is very resistant to drought.

Palatability: It is a palatable grass.

**Sowing time and rate**: It is grown during rainy season for higher establishment with 4-6 kg seed/ha.

**Sowing method**: Sowing to be done either by broadcasting or in line at 50 cm spacing. **Tolerance to salinity**: It has poor tolerance.

**Fertilizer requirements :** For higher forage production 120 kg N+30 kg  $P_2O_5$ /ha should be applied in mansoon season. Application of 120 kg N to be done in 4 split at 15 to 20 days intervals.

**Forage production :** The dry forage production ranged from 3-4 t/ha. However, the highest forage yield of 10 t/ha has been reported by Dwivedi et. al. (1980) with application of 90 kg N+40 kg  $P_2O_5$ /ha in C. fulvus cv. Mhow. The crude protein contain reported by them was 4.8-5.7% while calcium and phosphorus content ranged from 0.40 to 0.59% and 0.09 to 0.11%.

## Dichanthium annulatum (forsk.) Stapf.

Synonym: Andropogon annulatus (Forsk.)

Common names: Sheda grass (Australia), Lindi the Philippines, Kleberg blue-stem(United States), Pitilla (Cuba), Karad, Marvel grass in Maharashtra, Kail grass in Bundelkhand region of U.P. and Zinzwa grass in Gujarat.

Distribution: Tropical Africa to Southeast Asia, New Guinea and northern Australia.

**Description:** Tufted perennial and up to 80 cm (plate 3), the nodes bearded; leaves papillosepilose at least on the upper surface; first glume of the sessile spikelet not indurate,



PLATE 3: A STAND OF DICHANTHIUM ANNULATUM

or slightly indurate. Two to six racemes, some times more. Lower glume of sessile spikele with tubercle based hairs towards the tip, oblong, obtuse or truncate, keel not winged. Median nerve present, sheaths terete, longish (Bor, 1960). It differs from <u>D. caricosum</u> in having the first glume keeled, not winged, a medial nerve, and large membranous ligule (Dabadghao and Shankarnarayan, 1973). Ninety six percent of its roots end within a depth of 1 m. It differs from <u>Bothriochloa pertusa</u> in having no pitting on the glumes (Narayanan and Dabadghao, 1972) and from <u>Dichanthium sericeum</u> by the spikelets having a naked appearance due to the hairs being few or almost absent. The spikelets are also very blunt at the top (white, personal communication). Roots penetrate to 100 cm in alluvial soil at Varanasi, India, with a yield of 11275 kg/ha of oven dried roots.

Latitudinal limits: 8°28 N in India.

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Latitude range: It has a range of 250-1,375 m in India.

Rainfall requirements: Tropical and subtropical rainfall patterns. It is found mostly in India in the 500-900 mm rainfall regime (Dabadghao and Shankarnarayan, 1973). It persisted poorly in arid zone trials at Alice springs, Australia.

Drought tolerance: It evades or endures drought well (Whyte, 1968).

Tolerance of flooding: It survives short term flooding.

**Soil requirements**: It tolerates a wide range of soils but prefers black cotton soils in India and will not thrive in acidic soils.

**Tolerance to salinity:** It tolerates saline soils well and occurs on such soils in India in association with <u>Sporobolus marginatus</u>.

Fertilizer requirements: In India, the application of 22.75 kg N/ha to a natural pasture of

predominantly  $\underline{D}$ . annulatum increased the content of  $\underline{D}$ . annulatum and decreased Heteropogon contortus and Eremopogon. Rai (1990 a), obtained maximum dry forage yield (5.23 t/ha) with application of 120 kg N+30 kg  $P_2O_5$  ha. However, he found maximum net income of Rs. 271.9/ha at application of 30 kg N/ha.

Ability to spread naturally: Good.

Land preparation for establishment: A good seed bed is required for early establishment, but it will gradually colonize a rough seed bed.

**Sowing methods**: usually established from rooted slips in India, as seed collection is laborious and expensive. Rai (1987) reported that planting of seedling/rooted slips was found suitable material for better establishment of this grass as compared to seeds. It is sown in rows 60 cm apart with a similar distance between the plants, as they form large tussocks. However, Rai (1990 b) observed that there was no significant differences on establishment either seeds to be broadcasted or sown in line.

Sowing time, depth and rate: Sow at the commencement of the wet season. However, sowing in middle of July showed higher establishment and production as compared to middle of May and June (Rai, 1989). Sowing of seed at the rate of 4 kg/ha was found to be optimum for establishment and production of this grass (Rai, 1990 b). The optimum depth of sowing of this grass was found to be 0.4 to 0.8 cm as compared to surface (0 cm), 1.2, 1.6, 2.0 and 2.4 cm (Rai, 1990 c).

**Dormancy :** In India, Skerman and Riveros (1990) reported the filtrate of the rhizosphere fungus <u>Trichoderma viride</u> reduced the germination of <u>D</u>. <u>annulatum</u> seed from 90 to 77 percent.

**Growth rhythm:** It grows during the wet season from June to November in India and after harvest in November for hay. It provides spring growth from February to March, but this growth is stemy (Dabadghao and Shankarnarayan, 1973).

Compatibility with other grasses and legumes: It does not grow well in mixtures as it crowds out other grasses but it can be grown with various pasture legumes i.e. <u>S. hamata,</u>

M. atropurpureum, M. lathyroides etc. (Rai, 1988 a,b, 1990 d, 1992).

Response to defoliation: It forms on open turf under grazing and stands very heavy grazing (Dabadchao and Shankarnarayan, 1973).

Grazing management: It is not usually managed, but if over grazed, a <u>Dichanthium-Iseilema</u> grassland in Bellary, Mysore, India deteriorates first to <u>Bothriochloa</u> sp., then <u>Eremopogon</u> sp. and <u>Andropogon</u> sp. and finally to an inferior <u>Aristida</u> sp./<u>Andropogon</u> sp./<u>Eragrostis</u> sp. sward. Green matter production fell from 6000-10,000 kg/ha with <u>Dichanthium-Iseilema</u> to 200-1500 kg/ha with poor <u>Aristida</u>, <u>Andropogon</u> and <u>Eragrostis</u> association.

Genetics and reproduction: 2n= 20, 40, 60. It is quite a variable species.

Dry and green matter yield: At Bellary, Mysore, India, on black cotton soils a mixture of D. annulatum and Iseilema anthephoroides yields 6,000-10,000 kg/ha of green herbage. An average hay production of 3300 kg/ha can be expected from a good D. annulatum stand (Dabadghao and Shankarnarayan, 1973). For higher dry forage yield and better animal production S. hamata to be grown in association with D. annulatum (Rai and Verma, 1995).

Suitability for hay and silage: It is widely used for hay in India (Narayanan and Dabadghao, 1972).

Palatability: Good. Preferred to Cenchrus ciliaris in India.

Seed production and harvesting: It seeds heavily and in India seeds are hand collected.

Value for erosion control: Numerous grasses tested for stabilizing the bunds in the ravine lands of Gujarat, India, <u>D. annulatum</u> proved one of the best because of its elaborate root system and excellent ground cover. It has also proved useful for erosion control on 20° slopes.

**Economics:** On the black cotton soils of Bellary, Mysore, India, it is a climax species with Iseilema anthephoroides and is a palatable and nutritious species. It is a climax species along with Sehima nervosum over practically the whole of peninsular India and one of the most important grasses in the Dichanthium/Cenchrus/Lasiurus cover in semi-arid northern India (Dabadghao and Shankarnarayan, 1973). It is widely used in the Philippines for pasture improvement.

Main attributes: It has got wide adaptability, tolerance of alkaline soils and effective erosion control.

Main deficiencies: Its variability and its dominance of other grasses.

#### Panicum maximum Jacq.

**Common names :** Guinea grass (Australia, United States), Zaina, Pasto Guinea (Peru), gramalote (Puerto Rico).

Natural habitat: Grassland and open woodland and shady places.

Distribution: From tropical Africa, but introduced in many countries.

**Description:** A tufted perennial, often with a shortly creeping rhizome, variable 60-200 cm high(plate 4), leaf blades up to 35 mm wide tapering to fine point; panicle 12-40 cm long, open spikelets 3-3.5 mm long, obtuse, mostly purple red, glumes unequal, the lower one being one-third to one-fourth as long as the spikelet, lower floret usually male. Upper floret (seed) distinctly tranversly wrinkled.

Season of growth: Summer and Rainy.

Optimum temperature for growth: The mean range is 19.1-22.9°C.

**Minimum temperature for growth :** Mean temperature for the coldest month ranges from 5.4-14.2°C

**Frost tolerance**: It will not tolerate heavy frosts, but recovers from light frosts with the return of warm weather.

Latitudinal limits: 16.3-28.7° N and S.

Altitude range: Sea level to 2500 m.

Rainfall requirements: It requires a rainfall usually in excess of 1000 mm per year. With a summer dominance, cv. Gatton and Creeping Guinea do not tolerate very wet conditions.

Range 780-1797 mm.

Drought tolerance: It does not tolerate severe drought.

\*Tolerance to flooding: It does not tolerate waterlogging.

**Soil requirements**: It will grow on a large range of soils, but produces poor stands on infertile types. It is well adapted to sloping. It will tolerate acid conditions if drainage is good.

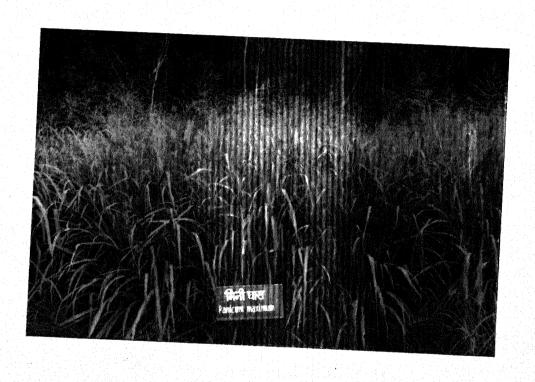


PLATE 4: A STAND OF PANICUM MAXIMUM

Tolerance to salinity: It has little tolerance.

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Fertilizer requirements: The optimum content of phosphorus in the dry matter was determined as 0.185 percent. Inoculation with Spirillum lipoferum increased yield by 480 kg DM/ha without nitrogen and 1021, 1690 and 1930 kg/ha with 20, 40 and 80 kg/ha, respectively (Skerman and Riveros ,1990). Phosphorus at 24 kg/ha and nitrogen at 137 kg/ha are required in north Queensland, but soil fertilizer experiments are required to diagnose needs on various soils. Hendrick concluded that a nitrogen levels above 45 kg/ha, phosphorus and potassium may become limiting to P. maximum in western Nigeria. It tolerates high aluminum.

Ability to spread naturally: It spreads slowly by seed, but needs fertile soil.

Land preparation for establishment: Well seed bed preparation is generally required for Guinea grass establishment.

Sowing methods: Drilling on the contour is small drill furrows and pressing in with press wheels gives an excellent stand. Sowing seeds at intervals of 0.6 m in rows 1.25 m apart is successful but laborious. In Sri Lanka, it has been found that close planting of P. maximum cuttings (with a spacing of 15x45 cm) increased yield. Transplanting of P. maximum seedlings is more reliable than that of root cuttings, especially if they have recently started to show new growth after rain. In Puerto Rico it is also generally sown by clumps of roots. One hectare will provide material for five hectares of planting.

**Sowing time and Rate**: Sowing is done in rainy season or in spring or early summer, so the pasture is established before the extreme heat of summer, at 3-6 kg/ha (1-2 kg for 'Hamil', 3.5-4.5 for 'common'.

Dormancy: The quality of the seed improves for some months after harvest.

Compatibility with other grasses and legumes: Guinea combines well with the legume centro (Centrosema pubescens) and this is a common pasture mixture for the wet tropics. In Brazil, 'Coloniao' Guinea centro and siratro are used successfully. Guinea and Stylosanthes guianensis is a successful mixture, Puero and glycine also combine well.

Response to defoliation: Guinea grass stands a good deal of defoliation but should not

**Response to defoliation:** Guinea grass stands a good deal of defoliation but should not be grazed or cut below about 30 cm for permanence.

Grazing management: In the wet tropics it is necessary to let this pasture become well-established before grazing so that it can compete with weeds. Guinea usually seed in autumn; do not graze a new pasture until after this seedling period. Guinea can not be grazed below 35 cm, or it will recover slowly. Adjust the stocking rate to maintain this height. Rotational grazing will give better control of pasture growth. Mowing or slashing is useful to control excess growth and weeds, but do not mow below 35 cm, and not after mid-autumn, as it will give slow regrowth and encourage winter weeds. Do not graze under extremely wet conditions, as trampling damages pastures growing in boggy ground.

**Genetics and reproduction :** The somatic chromosome numbers are 2n=18, 32,48. It is facultative apomict in which both apospory and pseudogamy occur. The amount of sexual reproduction varies from 1-5 percent depending on the variety.

⁴ Dry and green matter yields: At South John Stone, Queensland, cv. Makueni produced more than 60,000 kg DM/ha when 300 kg/ha of nitrogen was applied while in Puertico Rico 26,846 kg DM/ha was obtained with 440 kg N/ha, cut at 40 days intervals.

Suitability for hay and silage: It has been used successfully for silage at Mpwapwa, Tanzania, Brazil, Nigeria and Australia. It also makes useful hay in Thailand.

Palatability: It is very palatable.

**Seed production and harvesting:** Seed ripens unevenly, and is shed as it matures. In the Phillippines, highest seed yield (19 percent recovery) was obtained when the panicle had shed 40-60 percent of its spikelets, which occurred about 12 to 14 days from panicle emergence. Harvesting is usually done by direct heading.

**Seed yield :** Seed yield generally ranged from 48 to 156 kg/ha, however, 395 kg/ha from three cuts was obtained in Cuba.

Value for erosion control: Its great bulk aids in erosion control but its generally tussocky growth makes it less valuable than other species.

**Main attributes:** Its wide adaptation, quick growth and palatability, easy of establishment from seed and good response to fertilizers.

#### Pennisetum pedicellatum trin.

**Common names :** Annual kyasuwa grass (Nigeraia), Bara (Mauritania), Deenanath grass (India).

Natural habitat: A secondary weedy invader of disturbed sites, road edges and fallows.

**Distribution :** Native of north tropical Africa and India.

**Description :** A tall, annual, bunch grass, up to 1 m high (Plate 5) branches from the base and above, leafy. Leaves 15-25 cm long by 4-10 mm wide, flat glabrous. Racemes cylindrical, 5-12 cm long, dense-flowered, rachis glabrous, notched, outer bristles few, slender, short (about 3 mm long); inner bristles numerous (longest 9 mm) densely villous

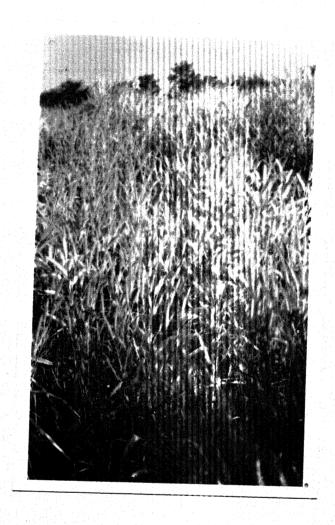


PLATE 5: A STAND OF PENNISETUM PEDICELLATUM

below the middle. Spikelets 4 mm long, usually solitary. It differs from <u>P</u>. <u>setosum</u> in having the inner bristles of the involucre densely villous while in <u>P</u>. <u>setosum</u> the inner bristles are laxly ciliate with long silky hairs.

Season of growth: Summer and rainy.

Optimum temperature for growth: 30-35°C.

Frost tolerance: It has little frost tolerance.

Latitudinal limits: 20° N and S.

Rainfall requirements: In Bihar, India, it grows on a rainfall of 1270 mm between June to September, from which it can produce seeds. The usual rainfall range is 500-650 mm.

Drought tolerance: It has good drought tolerance. It persists well in northern Nigeria with a dry season of seven months.

**Soil requirement:** It does best on fertile, loamy soils but with manuring, can grow in sandy soils. It can tolerate both acidic and alkaline soils (Narayanan and Dabadghao, 1972).

Fertilizer requirement: It responds well to added nitrogen.

Ability to spread naturally: It spreads rapidly by self sown seed.

Land preparation for establishment: It needs a well prepared moist seedbed.

Sowing methods: The seed is broadcasted or drilled in rows at 45 cm apart in India.

Sowing depth and cover: It is either surface sown or drilled at 1 cm.

Sowing time and rate: Just before the rainy season (May-July in India) at 1-2.2 kg/ha.

Compatibility with other grasses and legumes: It grows well in mixtures with Stylos,

Phaseolus mungo and Melilotus alba in India.

Response of defoliation: It can stands several cuts a year for green fodder.

**Grazing management :** It is generally used as a cut and carry green forage in India at ear emergence (80-90 days).

**Genetics and reproduction :** 2n= 36, 48, 54. There is a wide range of growth forms. It is strongly appraictic (Whyte, 1964).

Dry and green matter yields: At the Punjab Agricultural University, Ludhiana, India, four cultivars of P. pedicellatum yielded from 9.6 to 11.0 t/ha green matter compared with 5.7 t/ha from sweet Sudan grass and 3.6 t/ha from Sorghum. It is cut two or three times a season, first 80 days after germination and subsequently at 60 days intervals. It has also yielded good hay in Nigeria and Sierra Leone (Whyte, 1964). The dry forage yield of this grass can be enhanced upto 9.2 t/ha with improved variety like IGFRI-S-2808 as well as introduction of legumes (Dwivedi, et. al., 1982).

Suitability for hay and silage: It has been made into silage in Nigeria, Sierra Leone and India and also into hay.

Palatability: It is very palatable to cattle in India. It has a high leaf/stem ratio.

Seed production and harvesting: It seeds abundantly and matures very quickly in India.

Seed yields: Up to 2 t/ha (Whyte, 1964).

Value for erosion control: It is a valuable soil stabilizer in India.

Economics: In India it is a valuable grazing grass for sheep, goat and cattle (Bor, 1960).

It is also good as a short term hay and soil stabilizer. In northern Australia it is a weed.

Main attributes: Its early flowering, high tiller number, high leaf/stem ratio, low oxalic acid content, and palatability.

**Main deficiencies**: Being an annual it provides only short term grazing, can become a weed of cultivation.

## **REVIEW OF LITERATURE**

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## History of Allelopathy:

Allelopathy (root words: allelon and pathos) is derived from the Greek allelon, 'of each other' and pathos, 'to suffer': hence it means: the injurious effect of one upon another. The term denotes that body of scientific knowledge which concerns the production of biomolecules by one plant, mostly secondary metabolites, that can induce suffering in, or give benefit to another plant. The phenomenon could also be considered as a biochemical in eraction among plants. The concept suggests that biomolecules (specially term allelochemicals) produced by a plant escape into the environment and subsequently influence the growth and development of other neighbouring plants. The subject not only deals with the grass biochemical interactions and their effect on physiological process but also with the mechanism of action of allelochemicals at specific sites of action at the molecular level (Rizvi et.al., 1992).

The term 'allelopathy' was coined by Molisch in 1937 and his definition referred to both the detrimental and beneficial interactions among all class of plants, including microorganisms. This has led Rice (1984) to give the following definition of allelopathy: 'any direct or indirect harmful or beneficial effect by one plant (including micro-organisms) on another through production of chemical compounds that escape into the environment'.

Harper 1970) used the term interference to describe changes in the environment. Interference, thus includes the accepted competition which occurs for environmental resources, together with any allelopathic effect which may occur.

The recent upsurge of interest in allelopathy, with major volumes of collected papers

regularly published (Rice, 1984; Thompson, 1985; Putnam and Tang, 1986 a,b; Waller, 1987; Chou and Waller, 1983) has established the topic as one of biological significance, although the ecological impact of allelopathy remains a subject of debate.

Trans- cisciplinary studies suggest that the significance of allelopathy may be extended even further. Entomologists, for example, write of 'allelochemicals' in a context much wider than plant scientists concerned with allelopathy. Thus, Reese (1979)defines allelochemicals as 'non nutritional chemicals produced by one organism that affect the growth, health, behaviour or population biology of other species'. Behaviour-controlling chemicals semiochemicals are beginning to find a place in integrated pest management systems (Pickett, 1988), realizing a potential which has frequently been discussed in the literature.

We perceive allelopathy as one of the many stresses with which plants must cope in their environment. Recent data suggest that the responses to other stress factors, for example, invasion by viruses (Bassi et.al., (1986), or stress by heavy metals (Wierzbicka, 1987) are similar. To extend this concept further, there is evidence that chemicals identified with allelopathy may also affect other organisms, and that the responses of these organisms follow a common pattern (Lovett et.al., 1989).

It seems likely, giving the present state of knowledge, that allelopathy might best be regarded as a part of a complex network of chemical communication between organisms (Harborne, 1987) in which groups of chemicals compounds ellicit similar, quantifiable, responses from disparate organisms (Lovett, 1982).

#### Allelopathic Phenomena:

## Secondary effects:

Reports of allelopathic phenomena most frequently identify effects which are readily observed in the field or under controlled conditions. Delayed or inhibited germination and the stimulation or inhibition of root and shoot growth are often reported. Yet, the visible effects of allelopathy are merely secondary expressions of primary effects upon metabolic processes (Winter, 1961).

#### **Primary effect:**

Many possible primary effects on plant metabolism, affecting the majority of vital processes, have been suggested (Rice, 1984) but few have been rigorously investigated. Most attention has probably been paid to effects of allelochemicals on cell elongation and ultrastructure of root tips; for example, Lorber and Muller (1976). Koch and Wilson (1977) reported on the effects of allelochemicals on mitochondria but the total volume of work on primary effects remians small. Thus, in the five recent collection of papers cited, few focus mainly on this topic, although Waller (1989) notes that studies of allelopathy are moving 'quite rapidly' from practical considerations to molecular biology. Among recent contributions are a hypothetical sequence of action for the effects of phenolics. One of the most frequently reported groups of allelochemicals, proposed by Membrane function and interaction with hormones are included. However, supporting evidence is not abundant and there is a paucity of data on the primary effects of other important groups of allelochemicals, for examle, alkaloids. An examination of mono-and sesqui-terpenes as plant germination and growth regulators, again, points to the lack of evidence for elucidating primary effects (Fischer, 1986).

Most studies of primary effects have focused on early plant growth, a time of high metabolic activity but great susceptibility to environmental stress. Some weeds, for example, <u>Datura stramonium</u> L.(thornapple) (Lovett <u>et . al.</u>, 1981) release from the seed coat, compounds which have the ability to inhibit early growth of competing seedlings in their vicinity. Some crop plants, for example, <u>Hordeum vulgare</u> L. (Liu and Lovett, 1990), have a similar facility.

The chemicals involved in both these examples are alkaloids, compounds widely used in medicine and veterinary science and highly active, biologically. In thornapple and barley they appear to contribute to plant defence by causing disruption at the level of the cell (the primary effect) which is observed as impaird germination or reduced early seedling growth (the secondary effects).

The concept of germination inhibitors is due to Molisch (1922) and their presence in reproductive structures of plants such as fruits, seeds etc. has been a subject of discussion since then. According to Mayer and Poljakoff-myber (1982), Wieshner (1897), was the first worker to suggest that the seeds of <u>Viscum</u> sp. contain a germination inhibitor. Oppenheimer (1922) was among the first to study the problem experimentally. He tested among many other things the juices of tomato to determine whether they contain a germination inhibitor or not and concluded that they did indeed contain such an inhibitory substance. Later on Akkerman and Veldstra (1947) showed that caffeic, ferulic acids are the compounds responsible for inhibition of germination inhibitors as 'Blastocholines'.

Molisch (1937), the originator of the term allelopathy, was very much aware of the biochemical interactions between plants and animals, and between one animal and other

in addition to interactions between plants. As research progressed it became increasingly evident that there are many close relationships between various types of chemical interactions. Many of the same or related compounds are involved in interactions between plants and animals as pointed out by Whittaker (1970, 71). Whittaker and Feeny (1971) suggested that all interspecific chemical agents, other than used as food be called allelochemics or allelochemicals and the phenomenon as 'Chemical Ecology'. Harborne (1972, 1977 a) termed these chemical interactions as 'Phytochemical Ecology' or 'Ecological Biochemistry' and Numata (1978) as 'Allelobiology'.

In addition to setting limits of growth by inhibiting germination of other species, allelopathic sustances are also important in self inhibition (Evenari, 1961; Went and Sheps, 1969). This phenomenon has also been termed as auto-allelopathy. According to Went and sheps (1969), this is a factor of major importance in the survival of many temperate annuals by preventing the germination under adverse climatic conditions. Same is the case of desert plants when water supply is a limiting factor. It has been found that seeds of many desert annuals must be adequately leached by the rain before germination can occur. This not only serve to remove the seed coat inhibitors, but at the same time also assures growth only in the presence of an adequate water supply of soil moisture which permits the survival of the plants. The result is that once desert annuals germinate, they usually survive and form seeds. In other words competition is eliminated by germination control mechanism. Self inhibition also plays a role in population control. By preventing the germination of its own propagules under the already established plant, adequate spacing as well as more water availability is assured for the existing plants (Went, 1948, 49, 57, 74; Went and Sheps, 1969).

According to Beck and Reese (1976), allelopathy, production of phytotoxins, attractants, repellents, deterrents, antifeedents, germination inhibitors and toxicants are examples of allelochemic interactions.

Only organic compounds involved in allelopathic reactions have been considered as allelopathic agents and has been termed allelochemicals. According to Swain (1977) more than ten thousands such organic compounds are known to exist in plants. They have been termed 'secondary compound 'by Fraenkel (1959) and Whittaker and Feeny (1971), because they are produced and byproducts of primary metabolic pathways and have no recognised role in the maintenance of fundamental life processes in the organisms that synthesize these. Swain (1977) considered that the function of such secondary compounds are as chemical signals in ecosystem and there are documented example of the significance of such chemicals in communication between plants and other organisms. including the performance of defensive and even offensive function for the plant which produces them. Levin (1976) considered resistance to fungi, bacteria, viruses etc. as a basis for the presence of secondary compounds, particularly the phenolics. According to Lovett and Levitt (1981) allelochemicals are part of checks and balances that maintain relative stability and many responses to allelo-chemicals are, therefore, subtle having developed during evolution of the community.

Evanari (1949) gave a list of 121 species which produce inhibitor of germination of their own seeds or other test seeds. The same author in his summary has further stated "The presence of germination inhibiting substances in plants seems to be a wide spread phenomenon. They occur in all parts of the plant e.g. fruit pulp, fruit coat, endosperm,

seed coat, embryo, leaves, bulbs and roots". Since Evanari's review a considerable number of additional publications have appeared further extending the list of plants and plant parts containing inhibitors. Important contributions have been made in the identification of compounds involved in allelopathy with the advancement of scientific knowledge especially in chromatography and absorption spectroscopy (UV and NMR).

Germination inhibitors are also of wide occurrence in the juice of many other fruits viz., orange, lemon, straw berries, apricots etc. and has been identified as coumarins are derivatives of cinnamic acid, benzoic acid and other organic acids by Varga (1957 a, b,c,d,1958). He suggested that inhibitory activity was the result of the additive effect of a number of such compounds.

The role of germination inhibitors in dormancy and the ecological significance has been discussed by various workers viz., Barton, 1965 a,b; Wareing, 1965; Roberts, 1965; Wareing and Saunders, 1971; Kefeli and Kadyrov, 1971; Kollar, 1972; Taylorson and Hendricks, 1977; Mayer and Poljakoff- Mayber, 1982; Bewley and Black, 1985; Rice, 1984, 86; Lewak; 1984; Kefeli and Dashek, 1984; Kefeli, 1985; Fenner, 1985; and Putnam and Tang, 1986 a.

It is a well known phenomenon that barley (<u>Hordeum vulgare L.</u>) and oats (<u>Avena sativa L.</u>) exhibit dormancy when freshly harvested. The removal of hull permits the germination of the isolated caryopsis, so that evidently the hull exerts an inhibitory effect. It is also known that the hull contain water soluble inhibitory substances (Elliot and Leopold, 1953; Cook and Pollock, 1954) though Black (1959) provided evidences that in <u>Avena fatua Linn</u>, the inhibitory effect of hull is not primarily due to these substances, but to the fact that they prevent the leaching out of other inhibitors from the caryopsis. Khan

et. al. (1964) reported germination inhibitors from barley while Van Sumere et. al. (1958) isolated coumarins, hydroxy cinnamic acid and their derivatives as well as vanillic acid from barley husk.

Phenolic compounds reported from seeds and fruits includes simple phenol such as catechol and hydroxyquinone, phenolic acids such as p-hydroxy benzoic acid, salicylic acid, vanillic acid, protocatechuic, syringic, gentisic, gallic, ellagic acids, hydroxy cinnamic acid such as caffeic, ferulic, p-coumaric, sinapic, glycosides of hydroxy cinnamic acids, flavonoids and their glycosides such as anthocyanins, flavones, flavanols, flavanones, isoflavones etc.and tannins (Harborne, 1967; Bate-Smith and Metcalfe, 1957).

Varga and Koves (1959) identified several phenolic acids and gallotannins in dried fruit of 24 species of plants. Compounds involved in allelopathic activities have been reviewed from time to time (Rice, 1974, 84; Bhakuni and Silva, 1974; Robinson, 1974; Strobel, 1974; Gross, 1975; Thompson, 1985).

Mikkelsen and Sinha (1961) demonstrated the presence of a number of water soluble inhibitory substances in the hull of rice (Oryza sativa Linn.) e.g. vanillic acid, ferulic acid, p-hydroxy benzoic acid, p-coumaric acid and indole-acetic acids, which delayed the germination.

The possible ecological significance of germination inhibitors has been discussed by Evanari (1949, 57), who pointed out that the occurrence of germination inhibitors in the seed or fruit tends to result in sporadic germination over a period of time. According to Went (1949) in some desert species germination occur only after a certain quantity of rain has fallen and it appears that this requirement for a minimum rainfall is determined by the rain required to leach out the inhibitors.

According to Oppenheimer (1960), presence of germination inhibitors is an adptation to drought xerophytism.

Many types of phenolic compounds occur in fruits and seeds both as aglycones and glycosides (Feenstra, 1960, Harborne, 1964, 65 a, 67, 73 b, 79, 80; Harborne and Simmonds, 1964; Henis et. al., 1964; Harris and Burns, 1972). Phenolic compounds rarely occur in free state in living tissue. They are practically present in conjugated form, as water soluble glycoside (Harborne, 1979; Wollenweber and Dietz, 1981).

Regarding the possible role of germination inhibitors in seed dormancy, Wareing (1965) concluded "when we come to consider the possible role of inhibitors in seed dormancy phenomenon, the situation is more complex". While reviewing the relationship between seed dormancy and germination inhibitors in cereals, Roberts (1965) is also of the opinion that in some species there is good evidence that dormancy is largely controlled by germination inhibitors, however, in some other cases, the role of germination inhibitors in dormancy is obscure.

According to Van Sumere et. al. (1972) phenolics and coumarins and their derivatives are often reported as almost universally present inhibitors, which also act as germination inhibitors in seed husk, coats, fruits etc.

Germination inhibitors in fruit juices ensure that germination will only occur if the seeds are some way dispersed and this will occur if the fruit is decomposed or broken or if it is eaten by animals and the seeds subsequently excreted (Mayer and Poljakoff - Mayber, 1982).

Mayer ard Poljakoff-Mayber (1982) has reviewed the role of germination inhibitors in fruits, seeds etc. and stated "It must, however, be remembered that these resumed

function of inhibitors in fruits are by no means finally proven and infact they are very difficult to prove unequivocally. It is possible to interpret the observed fact differently (as already discussed). It is to be hoped that different approaches will lead to new lines of research into the probable biological and ecological function of inhibitors".

According to Rice (1984), inhibitors of preharvest seed germination may play an important role in preventing the germination of seeds before harvest or subsequent to cutting or stocking. Harris and Burns (1970) investigated the relationship between tannin content of the seed of hybrids of <u>Sorghum biocolor</u> and the preharvest germination of these hybrids. They found a strong negative correlation, indicating that tannins were good inhibitors of preharvest seed germination. Tannins like many other phenolic compounds are strong inhibitors of growth as well as germination. These are potent microbial inhibitors also.

However, in view of researches carried out especially since 1970 in the diverse field of chemical ecology, it has become increasingly evident that secondary compounds present in the seeds constitute the defensive mechanism and protect the seeds against the micro-organisms as well as from herbivorous predators. For example, according to Rice (1984) these secondary compounds act as preservatives: prevent the seeds from decaying after dispersal in a natural ecosystem and further stated (Chapter 8, Rice, 1984). "Probably one of the most critical points in the life cycle of many plants is seed germination. It seems surprising, therefore, that a little research has been done (allelopathy and preventation of seed delaying germination) in the past decade in this important area." Tang and Zang (1986) has also stated "seeds and fruits often contain pre-existing secondary metabolites

which inhibit microbial activity and seed germination".

Recent work carried out on the occurrence of secondary compounds in seeds has also revealed that secondary metabolites also act as feeding deterrent, repellent or antifeedents for a variety of phytophagous insects as well as herbivores and therefore, protect the seed from predators (Feeny 1975, 76; Bell, 1978, 81; Swain, 1977,79; Harborne 1979, 80; Bell and Charlwood, 1980; Mckey, 1979; Fox, 1981; Wilson, 1983; Janzen, 1983; Price et. al. 1984a and many others).

Recently it has been reported that secondary compounds present in the seeds also act as stimulators of germination and growth (Rice, 1986) as well as forms an allelochemic sphere around the germinating seedling, which (allelochemic sphere) adversely effect the germination and growth of co-germinating seeds resulting in the successful establishment in favour of the former.

The studies of secondary plant metabolites has become an exciting area of research (Krebs, 1985) and Price et. al. (1984 b). While discussing, "Is there a New Ecology" rightly stated "Ecology has moved from a strongly descriptive discipline to an experimental science". Descriptive studies of vegetation types, animal distribution have been replaced by studies of mechanisms. The transition from description to mechanisms, however, has not been accompanied by a whole sale move to a more rigorous application of the scientific method. Answers has been largely speculative at best. Had the scientific methods come into play adequately as the interpretive phase developed in ecology description would have provided the first link in erecting hypothesis about ecological mechanisms. Test of these hypothesis would have provided with some concrete information on the viability of some mechanisms and interpretations".

Parihar(1983); Parihar and Patil (1984, 86); Parihar (1985, 86b); Parihar and Kanodia (1986, 88) has also discussed the possible ecological significance of these phenolics present in the dispersal units of <u>Cenchrus ciliaris</u>, <u>C. setigerus</u>, <u>Dichanthium</u> annulatum and other range grasses.

Li, et. al. (1993) reported the lettuce seeds and eliolated seedlings (with hypocotyls about 3 mm long and roots about 5 mm long) were exposed to various concentration of trans-cinnamic acid, caffeic acid, ferulic acid, chlorogenic acid, abscisic acid (ABA), occumaric acid, m-coumaric acid, p-coumaric acid or coumarin. The growth of eliolated seedlings was inhibited by trans-cinnamic acid and o-, m-and p-coumaric acid at concentrations > 10<sup>-4</sup> M and seed germination was inhibited by those >10<sup>-3</sup> M. Coumarin inhibited seedling growth and seed germination at 10<sup>-5</sup> M or above. Chlorogenic acid inhibited seedling growth at > 10<sup>-4</sup> M but did not inhibit germination at 10<sup>-5</sup> to 5x10<sup>-3</sup> M. Low concentrations (<10<sup>-3</sup> M) of caffeic acid and ferulic acids promoted hypocotyle elongation, but higher ones (>10<sup>-3</sup> M) inhibited seedling growth and germination. These phenolic compounds and ABA had additive inhibitory effects both on seedling growth and germination. Inhibition (except that of coumarin on germination) could be reversed by applying caffeic acid or ferulic acid at concentration < 10<sup>-3</sup> M.

Bioassay, in the context of allelopathy, have been reviewed by Einhellig (1986). Probably the simplest forms of bioassay used in studies of allelopathy have been to quantify germination and/or emergence of seedlings, and to measure the length of the radicle or its equivalent. As defined by Winter (1961), although useful, such gross morphological data define only the secondary readily observable effects of allelopathy.

The main tool used in the small number of critical investigations of primary effects

of allelochemicals has also been the bioassay, but in more refined forms. Thus, the inhibition of mineral uptake in excised plant roots treated with phenolic acids are reported as being a consequence of the alteration of cellular membrane function (Balke, 1985), while phenolic acids, coumarins and flavonoids inhibit carbon dioxide dependent oxygen evolution in intact chloroplasts of spinach (Spinacia oleracea L.) and inhibit electron transport with mitochondria prepared from mung bean (Vigna radiata Roxb.) hypocotyls. Few published reports have combined bioassay with microscopy to elucidate primary allelopathic effects.

A bioassay has been developed capable of reliably assessing reduction in germination percentage and seedling length of small seeded plant species caused by exposure to minute quantities of these compounds. The germination and growth of lucerne (Medicago sativa ) cv. vernal, annual rye grass (Lolium multiflorum) and velvet leaf (Abutilon theophrasti) were evaluated against plumbagin, ( benzylisothiocyanate), cinnamaldehyde, coumarin, junglone and nigericin. Cinmethylin was selected as a comparison standard. Each phytotoxin, dissolved in a suitable organic solvent, was placed on water, agar in small tissue culture wells. After the solvent evaporated, imbibed seeds were placed on the agar, after 3 days, germination percentages and seedling lengths were measured. Compared to a commonly used filter paper procedure, this modified agar bioassay required smaller quantities of seed for comparable results. This bioassay also readily permitted the measurement of seedling length, a more sensitive indicator of phytotoxicity then germination seedling length decreased sigmoidally as toxin concentration increased longarithmically. Phytotoxicity was a function of both compound rye grass seedling by 90-100%, where as that of alfalfa and velvet leaf as inhibited only slightly. The

agar bioassay facilitated the rapid and reliable testing of slightly water soluble compound, requiring only minute quantities of each compound to give reproducible results (Dornbos and Spencer, 1990).

The occurrence of germination inhibitors in the propagative bodies (dispersal unit) of grasses is a common phenomenon. Therefore, inhibitors removal by soaking the seeds in water or placing them in activated charcoal beads before sowing has been recommended by various workers viz., Ballard (1964), Chippindale (1933) showed that soaking and redrying improve the germination in <a href="Dactylis glomerate">Dactylis glomerate</a> Linn. and this effect was evident in hulled grains and not in dehulled ones. Aqueous extract from the hull of dormant crab grass (<a href="Digitaria sanguinalis">Digitaria sanguinalis</a> (L) Scop.) inhibited the germination of nondormant seeds (Delouche, 1956). The inhibitory property was not destroyed by boiling the extract for 30 minutes. Similarly extracts prepared from non dormant seeds were not inhibitory. Kollar and Negbi (1959) showed that the dispersal unit of <a href="Oryzopsis miliaceae">Oryzopsis miliaceae</a> contained germination inhibitor which was essential to be leached before the germination.

Ching and Foote (1961) also found water and ethanol soluble growth inhibitors in the extract of dormant seeds of wheat (<u>Triticum vulgare</u>) and it was postulated that loss of dormancy was due to the oxidation of these inhibitors. Miayamoto <u>et. al.</u> (1961) also isolated four types of water soluble inhibitors from the seed coat of wheat, which possibly inhibited the germination of isolated wheat emryos. After three weeks of harvest it was seen that there was a loss of inhibitors since the dormancy decreased with the period of storage. It may be possible that these germination inhibitors play a role in dormancy mechanism.

In grasses also like some cereals, removal of glumes facilitates germinations which

has been attributed to the removal of germination inhibitors present in caryopsis enclosing glumes etc. Though some authors attribute the effectiveness of this treatment to the removal of structures constituting a barrier to oxygen diffusion to embryo (Carr. 1965). Many workers viz., Lahiri and Kharbanda (1962,63); Ahring (1963); Mott, (1974); Martin (1975); Hagon (1976); Pandeya and Jayan (1978); Parihar et. al. (1984, a,b) also observed enhanced germination of caryopsis as compared to diaspores. Lahiri and Kharbanda. 1962 and 63; Pandeya and Jayan, 1978 and Parihar and Kanodia, 1984 also recorded inhibition of germination by glume extract/spikelet leachate confirming inhibition of germination due to inhibitors. Lahiri and Kharbanda (1962) believed these inhibitors to be coumarins in Cenchrus ciliaris, C. setigerus and Lasiurus sindicus. However, subsequent work by Parihar and Patil (1984); Parihar and Kanodia (1984 and 86), demonstrated the presence of phenolic onium-ions, cyanidin glycoside and cinnamic acid derivatives in the caryopsis enclosing glumes of Cenchrus ciliaris, C. setigerus and Dichanthium annulatum. Parihar and Patil (1986) also demonstrated in inhibition of germination and inhibition of root and shoot growth of grasses in isolated phytotoxins by bioassay studies.

Panicum virgatum establishment from seed was limited by current years growth of Cenchrus longispinus in the Nebraska sand hills. Stand reduction was greater than in other warm-season grasses sown at the same time, indicating possible allelopathy. Fresh C. longispinus plant material was extracted with distilled water for 24 h. Root, shoot and whole plant leachate collected between the vegetation and culm elongation stage and whole plant leachate from vegetative or mature plants was used, P. virgatum germination was not influenced by root, shoot or whole plant leachate. However, leachate reduced the length of the primary root and increased shoot length. Generally, the response was greater

with vegetative <u>C</u>. <u>Icngispinus</u>, compared with mature and at the higher leachates concentration. Whole plant leachate from vegetative <u>C</u>. <u>Iongispinus</u> reduced <u>P</u>. <u>virgatum</u> germination cor pared with mature plant leachates (Roder, <u>et</u>. <u>al.</u> 1988).

According to Takahashi, et. al. (1988) 6 grass and 3 legume species were grown in sannd and the leachate, including the root exudate, from each species was applied to the 9 species grown in separate pots. The growth of all species was reduced by the addition of leachate, Lolium perenne causing the greatest inhibition and in Medicago sativa the least. The growth of most of the species, particularly M. sativa and Trifolium repens, was inhibited more by grass species leachate than by legume species leachate. The growth of Trifolium hybridum, T. repens and L. perene was inhibited more by their own leachate then that of other species. All the other species showed the opposite response.

In germination studies with seeds of the lettuce, namely white Bostan and aqueous extract of  $\underline{V}$ . sativa, obtained from the aerial parts, was applied to filter paper at concentrations of 100, 75, 50, or 25%. Control was treated with water. The effects on seed germination, length of the primary root and seedling dry weight were determined over 96 h. At 75 and 100% concentrations, the seeds did not germinate and decomposed rapidly. The more dilute extracts delayed germination and checked root growth (Medeiros and Lucchesi, 1993).

Laboratory and green house studies were conducted by Chung and Miller (1995 a), to determine the allelopathic potential of nine grasses to alfalfa (Medicago sativa) on germination and seedling growth. Alfalfa seeds were germinated in aqueous extracts of

nine grasses, using distilled water as a control. Measurement were taken to determine the effect of extracts on germination, seedling length, and weight. Alfalfa germination ranged from 64% for Festuca arundinacea extracts to 91% for the control. Total alfalfa seedling length was reduced by 39% for grain sorghum extracts. Dry weights of alfalfa cotyledons, hypocotyls and radicles were reduced significantly by several grass extracts. Bromms inermis, Dactylis glomerata and grain sorghum extracts were more inhibitory than other grass extracts. Alfalfa seedling emergence and survival percentage was affected by various grass root residues. Pheleum pratense extracts caused the lowest survival percentage of 59% compared with the control of 88%. Agrostis gigantia and Phalaris arundinacea extract has no effect on alfalfa seedling emergence and survival.

b) to investigate the allelopathy potential of <u>Chenopodium album</u>, <u>Setaria faberii</u>, <u>Amaranthus retroflexus</u>, <u>Abutilon theophrasti</u>, <u>Digitaria sanguinalis</u>, <u>Crisium areense</u> and <u>Polygonium aviculare</u> on germination and seedling growth of lucerne. The weeds were collected at their vegetative stage, and the shoots and roots were dried at room temperature. Extracts were prepared in distilled water and germination trials were conducted with lucerne seeds. Results showed that extracts obtained from shoots and leaves resulted in a greater allelopathic effect on lucerne germination than root extracts. Shoot and root extracts of all the weed species resulted significant inhibition of germination, seedling weight, vigour and germination rate of lucerne. <u>A. theophrasti</u> resulted in the greatest inhibitory effect, whilst <u>D. sanguinalis</u> had the least effect. Further germination trials were conducted using 0.0,0.5 10.0, 15.0 and 20% concentration of <u>A. theophrasti</u>. Results showed that lucerne seed germination, seedling weight and seedling

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length were inversely proportional to the A. theophrasti extract concentration. Dried extracts were more inhibitive than fresh extracts. Trials were conducted to assess the allelopathic effects of dried lucerne residues mixed into vermicutile on the germination, shoot length and root length of the crops such as Perilla frutescencs, Sehima nervosum and Plactycodon grandiflorum and the weeds Digitaria sanguinalis, Setaria viridis, Sigesluckia pubencens, Amaranthus lividus and Solanum nigrum. Lucerne residues were added at concentrations of 0.1, 1.0, 5.0, 10.0 and 20.0%. The above crop and weed species were significantly inhibited as the concentration of lucerne residues increased; concentration 10.0% resulted in 80.0% inhibition of germination and growth in the test species (Yu, et. al. 1995).

According to Chung and Miller (1995 c), then allelopathic effects of various alfalfa (Medicago sativa) plant parts the soil in which alfalfa was grown, on alfalfa germination and seedling growth were investigated. Aqueous extracts of alfalfa leaf, stem, flower, seed and root parts were made to determined there effects on germination and dry weights of hypocotyl, radicle and total length of 5 days old alfalfa seedlings over a range of extract concentration. Soil samples from around alfalfa plants at the vegetative and reproductive stages were compared with sterilized and non sterilized soil formerly sown with alfalfa, hairy vetch (Vicia villosa) and winter rye. Increasing the aqueous extracts concentrations of separated alfalfa plant parts significantly inhibited alfalfa germination, seedling length and weight. Radicle length was more sensitive to extract source than seed germination or hypocotyl length. Based on 5 days old alfalfa radicle length growth and averaged across all extract concentrations, the degree of toxicity of different alfalfa plant parts and soil from around alfalfa was classified in order of decreasing inhibition as follows: leaf, seed,

complete plant mixture, soil, root, flower and stem. Leaf extract caused a 48% decrease in water uptake by alfalfa seed. Soil in which alfalfa had previously grown was the most inhibitory to alfalfa growth after 25 days of growth compared with soil where witner rye or hairy vetch had previously grown. Inhibitory effects were greater for soil collected around alfalfa grown at the reproductive than the vegetative growth stage. It is suggested that alfalfa autotoxicity may result from a release of one or more water soluble compounds from alfalfa leaf tissue.

Studies on long term storage of grass seeds were conducted by Ackigoz and Knowles (1983) reported that seed of crested wheat grass, intermediate wheat grass and smooth brome were stored for 20 years, under various conditions. Temperature was a major factor affecting success with viability inversely related to storage temperature. At -7°C and -18°C, viability of 80-90% were shown after 20 year storage. Drying seed for 7-5 h at 60°C prior to storage gave little improvement over undried seed stored with 8% m.c. Plastic bags were as good as glass jars with screw top lids although plastic bags were less effective in excluding moisture. It was conducted that adequate germination could be obtained after 25-30 yrs of storage.

Parihar, et. al. (1984 b) studied the effect of age (storage) and removal of glumes on germination of Cenchrus ciliaris Linn. He reported that spikelets and dehulled seeds of 10 C. ciliaris cv.(cultivar) were stored for upto 4 years and their germination was tested at 1 year intervals. The germination percentage of seeds in spikelets was highest (average 38.8-39.1%) after 1 and 2 years of storage. Germination percentage of the dehulled seeds was markedly higher than that of seeds in spikelet and was highest (75.5%) after 1 year of storage.

The effect of seed storage on germination of different tropical grasses conducted by Matias and Bilbao (1985) revealed that all cultivars P. maximum had different reactions to storage. Sil-1-127 and the local ecotype showed highest germination after 2 months (12.54 and 9.6%, respectively), after this germination percentage dropped. Likoni and Australian showed highest germination at harvest (15.91 and 9.05%, respectively). Makueni showed highest germination at 6 months (25.89%) storage had a significant effect on C. gayana, with highest germination at 4 months (13.46%) where as in C. ciliaris germination was always low (1.5%) with no significant difference between different storage periods. Germination in S. bicolor decreased significantly from 66.53% at harvest to approximately 40% after a year.

Seed of <u>C</u>. <u>ciliaris</u> clones were started in butter paper bags under ambient conditions (Mean max. temp. 29.4-34.9°C, 77-90% RH) for 21 months and their germination was tested at 3 month intervals. Seeds of introduced clones, Anjan, FC 3108 and FS339 gave the highest germination (89, 73 and 68%, respectively) after 12 months storage and these of a local cultivar (cv.) (40%) after 18 months storage (Selvaraj and Ramaswamy, 1986).

Germination of the salt-tolerant grass <u>Diplachne fusca</u> was tested by Morgan and Myers (1989). They collected fresh seed of <u>D</u>. <u>fusca</u> at 2 locations over several years of dormant. Dormancy was not broken by scarification, but gradually broke down during airdry storage, indicating an after-ripening period of at least 1 year. Bract removal increased germination percentage and rate and significantly interacted with per soaking to increase germination percentages. When seeds were pre-soaked but to decrease them when soaked at 10°C, subsequent drying increased germinability. The germination of germinable stored seed at various temperature. Regimes (Combinations of day and night

temperature between 11 and 31°C) was assessed on a therogradient plate. The germination after 21 day was greatest (40-49%) at high temperature when both day and night temperature were between 24 and 31°C, either constant or alternating). Germination was completely inhibited when both day and night temperature were ≤18.5°C. Germination percentage was more strongly correlated with night than with day temperature. Seeds for which dormancy has been broken by scarification were capable of germinating at lower temperature (19% germination at 11°C) than stored seeds. From the observed temperature dependence of germination and the mean daily maximum and minimum temperature recorded at Tatura and Deniliquin, (latitudes 36° 26' S and 35° 32' S respectively), it was suggested that germination in the field, in the Riverain plain of SE Australia, would be limited to the summer months (Dec.-Feb.).

Scarified and non-scarified <u>Eragrostis lehmanniana</u> seeds from 7 seed lots were germinated over a water potential range of 0 to -1.16 M Pa. Six of the seed lots were harvested ≤ 1 year before the germination tests. Results showed that mechanical scarification increased total germination and germination rate. Mechanical scarification reduced variability among seed lots for germination rate but increased variability for total germination. Total percentage germination was least in the most recently harvested seeds in all treatments. It is concluded that rapid germination hypothesis may be valid for <u>E</u>. <u>lehmanniana</u> as long as seed numbers are not limiting. Of the scarified seed that germination above a water potential of -0.4 M Pa, at least 10% did so between days 1 and 2 of the study (Hardegree and Emmerich, 1991).

The effect of mechanical scarification on germination and seedling emergence of neoteric (1 to 18 month old) switch grass was studied by Nancy and Boe (1991).

Scarification for 15 or 30 second in a Forsberg cylinder scarifier significantly increased 14 day germination percentage for 1 to 5 month old seed of 5 cultivars. The magnitude of increase in seedling emergence due to scarification varied across cultivars. Four month and eighteen month old seed lots of 'Sun brust' and a North Dakota ecotype (NDE) exhibited significant increase in germination and seedling emergence after scarification. Scarification increased overall mean germination percentage for 3 lots of 'Sun brust' and 2 lots of NDE by 73%. Field studies are needed to determine the usefulness of mechanical scarification as a preplant treatment for neoteric.

Dormancy factors that contributed to slow germination and poor stand performance in Paspalum notatum were studied. Several dormancy releasing treatments were ineffective. The importance of the lemma was established by excising parts of the seed covers. Removing the second glue and sterile lemma did not reduce dormancy. Removing pales resulted in significantly improved germination but still not as rapid nor as complete as removing the lemma. Germination was observed to occur by the coleorhiza protruding through an opening in the lemma caused by the separation of fibres immediately above the embryo. Aging the seed increased germination and the number of seed with visible seperated fibres. A dormancy mechanism is proposed in which water uptake and the expansion of the embryo are restricted untill an opening occur in the lemma (West and Marousky, 1989).

Weaver and Jordan (1985) reported the effects of various treatments on germination rates of <u>Eragrostis lehmanniana</u>, <u>E. trichophora</u>, <u>E. curvula</u> variety <u>conferta</u>, <u>Panicum antidotale</u> and <u>Atriplex canescens</u> seeds. Rates were approximation of time of 50% germination and seed treatments included application of KNO<sub>3</sub>, Ammonium nitrate, GA<sub>3</sub>

and heat desiccation. Germination rates could be increased, but treatment effects were not uniform between seed lots within a species or amongst species. Desiccation at 70°C for 24h was very effective in increasing germination rates of <u>E</u>. <u>lehmanniana</u> and <u>E</u>. <u>curvula</u> variety <u>Conferta</u> seeds.

Studies on dormancy of seeds of <u>Melanocenchrus jacquemontii</u> showed dormancy for one month. A water soluble inhibitors also develops in hot months which becomes deactivated in July by rains or if the narrow range of temperature is available. Seeds Jose viability after 18 months of dry storage (Pathak <u>et al.</u>, 1976).

The effect of five growth regulators and three chemicals was studied on the germination in behaviour of <u>Parthenium hysterophorus</u> seed by Dagar <u>et.al.</u> (1977). He reported an inhibitory effect at all the five concentrations of IPA and NAA and four higher concentrations of 2, 4-D and interaction of GA<sub>3</sub> with IPA and 2, 4-D at 100 and 200 ppm was observed . 10 and 25 ppm of IAA also showed retardation in germination. Higher concentrations of IAA showed normal effects while in rest of the cases the effects were more or less promoting.

Seed germination studies were performed in petridishes lined with filter paper with distilled water in continuous light with many arid zone species. Treatment of acid scarification, continuous washing for 1-5 days and seed coat removal were used to enhance germination. Most of the seed possessed hard coat dormancy and some contained germination inhibitors. Germination ranged from 0-100% and variety of dormancy mechanism adaptive to the arid environment were found. Alysicarpus monilifer,

A. vaginalis, Aristida adscensionis, Eragrostis ciliaris, showed 1-20% germination,

Dactyloctenium acgypticum, Digitaria adscendens, Eragrostis tremula, Tragus biflorus

showed 20-50% germination and <u>Chloris virgata</u>, <u>Cyamopsis tetragenoloba</u> showed 100% germination (Bansal and Sen, 1981).

Germination of <u>Brachiaria decumbens</u> was shown to be controlled by two dormancy mechanisms. Primary dormancy was variably expressed in freshly harvested seed and over come by "affer ripening" during storage of upto three months. Long term dormancy may be due to mechanical restriction imposed by the seed coat and to inhibition of O<sub>2</sub> diffusion due to the closely appressed, hard shiny Palea and Lemma structure enclosing the caryopsis. Removal of these structure by hand allowed germination percentage up to 100 in naked caryopsis. Impermeability of the seed coat declined with time in storage upto one year. Germination of intact stored seed reached 40% to 55%. Further storage at 10°C and 29% RH upto 4 &1/2 years did not result in increased germination in intact caryopsis, although viability was maintained at 80% to 90%. Scarification in concentrated H<sub>2</sub>SO<sub>4</sub> for 20 minutes increased germination of stored seed to 72% (Whiteman and Mendra, 1982).

Fulbright, et. al. (1983) studied in a green house and found that germination of seed of <u>Stipa viridula</u> was highest (50%) when temperature was at constant 20° or 20/15° C (16/8 hr periods), in darkness, prechilled or treated with GA and the lemma and palea were clipped with a razor blade.

Gonzalez and Torriente (1983) reported that stored Guinea grass seeds were treated with 0, 0.1, 0.2 or 0.3% KNO<sub>3</sub> after 0, 1, 2, 3, 4 or 6 months. There was a significant interaction between KNO<sub>3</sub> and storage time on percent germination, germination energy percentage, dormancy and death. The highest germination of 46.09% occurred after 2 months storage with 0.2% KNO<sub>3</sub> compared with 37.9% without KNO<sub>3</sub> and 2.88% at the start of the experiment, germination energy (40.64%) was also greatest with this

treatment. Percentage dead seeds and abnormal germination increased with storage while germination energy decreased.

Effect of substrates and scarification methods on seed germination in buffel grass (Genchrus ciliaris cv. Biloela) was studies by Vieira Neto and Aragao (1984). Authors used sterelized sand or filter paper, the germination percentage of C. ciliaris cv. Biloela stored for 7 months was not significantly affected by 24 M. Sulphuric acid treatment for 30 minute. Treatment with ethanol for 10, 20 or 30 minute or with boiling water after freezing for 2-5 or 1-25 hr reduced percentage germination. Germination ranged from 0% in both substrates with boiling water after freezing for 1-25 hr. to 38-63% in sand after acid treatment.

Parihar and Rai (1985) reported that seeds of 9 grasses collected in 1974 and stored in polythene bags at room temperature were studied for viability. The minimum period of seed viability was at least 48 months for Cenchrus ciliaris and Chrysopogon fulvus, 60 months for Cenchrus setigerus, Heteropogon contortus and Sehima nervosum and 84 months for Bothriochloa pertusa and Bothriochloa intermedia. Germination of seeds of Dichanthium annulatum was consistently good for 4 years of storage and decreased in the 5th years. Panicum antidotale gave 19% germination after storage for 6 years.

Rodrigues, et. al. (1986) studied the effects of different methods of breaking seed dormancy of <u>Brachiaria humidicola</u> (Rendle) Schweickerdt. Washed and unwashed <u>B</u>. <u>humidicola</u> seeds were chemically (KNO<sub>3</sub>, GA<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>) or mechanically scarified and subjected to a constant (30°C) or alternating (20/35°, 16 hr/8 hr) temperature regime in the presence or absence of light. None of the dormancy breaking methods were successful

at 30°C. Treating washed seed with gibberellic acid followed by an alternating temperature regime was the most effective dormancy breaking method (51.5% germination). Sulphuric acid and light treatments were ineffective for breaking seed dormancy.

Seeds of 3 important Somali rangeland grasses (Cenchrus ciliaris, Dactyloctenium sindicum and Sorghum arundinaceum) were scarified by rubbing with sand paper and soaking in hot water or sulphuric acid. Seed germination of C. ciliaris was greatest (41%) in the hot water treatment and lowest (10%) in the sulphuric acid treatment. Both D. sindicum and S. arundinaceum germination was highest with the rubbing treatment (41 and 55%) and lowest with the sulphuric acid and hot water treatments (14 and 24% and 22 and 22%), respectively. The scarification treatments had not influence on the mean germination times of the 3 species except for sulphuric acid, which increased D. sindicum mean germination time from 6-2 days in the untreated controls to 10-3 days (Barker and Abdi, 1988).

Dormancy in freshly harvested seeds of <u>Panicum maximum</u> cv. PGG-19 was studied by Basra, <u>et</u>. <u>al</u>. (1990) to know the inhibitory influence of husks. Mechanical or acid dehusking markedly increased the germination percentage. Germination was further augmented when the acid dehusking was followed by soaking seeds in KNO<sub>3</sub>, GA<sub>3</sub> and or phthalimide. KNO<sub>3</sub> in combination with either GA<sub>3</sub> or phthalimide was the most effective. Phthalimide effectively mimicked the effect of GA<sub>3</sub>.

Toledo and Carvalho (1990) studied quantity of KNO<sub>3</sub> solution and the germination of 3 species of <u>Brachiaria</u> seeds. The seeds of <u>B. decumbens</u>, <u>B. brizantha</u> and <u>B. ruziziensis</u> were germinated on 2 sheets of paper wetted with 6, 12 and 20 ml KNO<sub>3</sub> or on

1,2 or 3 sheets of paper wetted with 6,12 and 18 ml KNO<sub>3</sub>, respectively. Germination of <u>B</u>. <u>decumbens</u> was not affected by substrate treatment. Germination of <u>B</u>. <u>brizantha</u> decreased with 20 ml KNO<sub>3</sub> on 1 sheet on paper and with 16 ml KNO<sub>3</sub> on 3 sheets of paper. <u>B</u>. <u>ruziziensis</u> germination decreased with 16 ml KNO<sub>3</sub> on 1 sheet and with 16 ml on 3 sheets of paper.

Burbano, (1990) studied the effect of chemical scarification and storage on seed quality in Centrosema species. Seeds of C. brasilianum cv. CIAT 5234, C. acutifolium cv. CIAT 5277 or C. macrocarpum cv. CIAT 5713 were subjected to several scarification treatments with 100 ml H<sub>2</sub>SO<sub>4</sub>/kg seeds and storage for 1-19 months in laboratory (22°C, 88% RH) or cold room (18° C, 50% RH) conditions. The percentage of normal seedlings was generally greater and the percentage of hard seeds was always lower with than without scarification before storage in all species. After 19 months storage with scarification 30 d before quality evaluation, the percentage of normal seedlings was greater with storage under cold room conditions, the percentage of hard seed decreased with increasing storage duration. After 19 months storage in laboratory conditions, germination and percentage emergence were greater without than with scarification 3 d before showing in C. brasilianum and C. acutifolium. Germination and emergence of C. macrocarpum after 19 months storage in laboratory conditions and of all species stored in a cold room were greater with than without scarification 3d before sowing. Emergence and germination were greater under cold room than laboratory storage conditions and were generally greatest with scarification every 30 d.

Quantities of KNO<sub>3</sub> solution and the germination of P. maximum Jacq. seeds were

studied Yoledo, et. al. (1994). Recently harvested seeds of 5 cultivars of P. maximum were germinated in the presence of 12, 16, or 20 ml of 0.2% KNO<sub>3</sub> solution either immediately or after storage under ambient condition at intervals of 4 months over a 2 years period. Germination percentage was significantly higher (28.5-78.4% germination) in the presence of 12 ml KNO<sub>3</sub> solution than at higher volumes (cultivar) cv. Centenario was the most sensitive and cv. Tobiata the least sensitive to excessive amount of KNO<sub>3</sub>.

In a laboratory experiment Khandelwal and Sen (1994) studied the effect of soaking seeds for 24 h in 50-1000 ppm KNO<sub>3</sub> on germination of fresh and 1 year old seeds of <u>Eragrostis ciliaris</u>, <u>E. tremula</u> and <u>E. poaeoides</u>, (<u>E. minor</u>). No seed germination was recorded for <u>E. poaeoides</u>. In <u>E. ciliaris</u> germination was highest (36.7%) in 1 year old seeds soaked ir 1000 ppm KNO<sub>3</sub> where as in <u>E. tremula</u> it was highest (96.7%) in 1 year old seeds soaked in 50 ppm KNO<sub>3</sub>.

Toledo, et. al. (1995) studied the seeds of P. maximum cultivars were treated with sulphuric acid and germinated immediately after treatment in August, 1991 or at intervals upto April, 1993 during which time they were stored under ambient conditions. Acid treatment did not improve germination, independent of storage duration.

# MATERIAL AND METHODS

# MATERIALS AND METHODS

#### Research Site:

## a) Location and Topography:

Present investigation has been conducted in Department of Botany, D.V. Post-Graduate College, Orai (Jalaun) which is situated at latitude 25° 59' N, longitude 79° 37'E and is about 125 m above mean sea level in Bundelkhand region as well as Indian Grassland and Fodder Research Institute, Jhansi located at 78° 35' E longitude, 25° 7' N latitude and 275 m altitude from the mean sea level the northern part of Bundelkhand region.

The region comprises seven districts of Uttar Pradesh viz., Jalaun, Hamirpur, Mahoba, Banda, Chitrakut, Jhansi and Lalitpur and five districts of Madhya Pradesh viz., Datia, Tikamgarn, Chhatarpur, Panna and Sagar. The region is naturally bounded by the river Yamuna in the north, range of Vindhyan Plateau in the south, river chambal in the north-west and Panna-Ajaigarh ranges in the south-east.

The region has undulated topography that tends to become into a perfect level plain towards north. About 1/3 northern part of the region is monotonously flat. Every where it shows gentle undulating surface occasionally flat topped surface. The main rivers namely the Betwa, Dhasan and Ken enter the alluvial plain in the north resulting in erosion on a very large scale to form some of the most extensive and fantastic ravine land.

Bundelkhand plain is also known as Trans Yamuna plain and is topographically devisible into three east-west running belts i.e. southern, central and

northern belts. The first belt narrower than the others and is confined along the bank of Yamuna in the form of high ground which represent the level of the ancient flood plain but which at present is badly cut up into deep ravines. Thus, the area in general exhibits subdued topography.

# ুb) Geology and Soils :

The common rocks of the area are sand stones, lime stones and shales. The peculiar features of geomorphic interest are the long narrow serrated ridges termed as quartz, reefs and dolorites, dykes in this region. In the north-west and north-east, the geological system is covered by Ganga and Yamuna alluvial deposities in the form of an embayment.

Soil of Bundelkhand region may be conveniently grouped into the following categories (Regional Geography of India, 1960).

- 1. Upland soil (rocky soil)
- 2. Low land soil- black (mar, kabar), red and yellow (parwa, rankar) soil.
- 3. River line soil (kachhar and tarai):

The most important soil groups of Bundelkhand are found in the northtern low land.

These are mar, kabar, parwa and rankar, formed partly in situ and partly by transporting agencies chiefly the streams.

Mar is calcarious soil predominantly blackish in colour mixed with lumps of Kankar and hence friable and aerated. Kabar on the other hand is highly diffused soil and is similar to mar in many physical characteristics. Parwa the best known variety of degraded red and yellow soil groups is well aerated, friable and receptive to irrigation and favourable

for various types of crops. Rankar is associated with flood plains subjected to gully and erosion so that calcium nodules are exposed at the sloping surfaces, rendering them unsuitable for cultivation. Thus on the basis a number of soil samples study, the soil is of medium textured and sandy loam to loam. The colour of the soil is light olive brown or olive brown which is slightly alkaline in reaction.

# c) Climate and Vegetation :

The climate of the area is a dry sub-humid, tropical monsoonic with a year divisible into three seasons namely rainy (July-October), winter (November-February) and summer (March-June). The annual temperature is uniformly high over 25° C but the mean monthly values vary considerably (13.9° C mean minimum to 34° C mean maximum). The mean total annual precipitation is 1169 mm of which about 80% falls between July to October.

Bundelkhand region can be considered as an ecologically degraded area having about 0.7 million ha area occupying central position in the country. The original vegetation cover of the area has almost been removed for inhabitation and cultivation. Out of total area about 7.2% is under mixed dry deciduous type of degraded forests. <u>Butea monosperma</u> (Dhak), <u>Salmolia malbarica</u> (Semal), <u>Boswellia serrata</u> (Salai), <u>Acacia nilotica</u> (Babul) and <u>A. catechu</u> (Khair) are the dominant trees of natural vegetation. Apart from these <u>Balanites aegyptica</u> (Hingota), <u>Carrisa carandus</u> (Karondha) and <u>Capparis aphylla</u> (Karil) shares a good proportion of the floristic composition of the vegetation. Scrubs and grasses represent the secondary growth throughout the region. Besides it, various forest tree plantations were introduced during past three decades under afforestation programme by the Government of India.

## Seeds( dispersal units) Collection and Germination:

Mature seeds (dispersal units) of the five range grasses were collected from the Central Research Farm of Indian Grassland and Fodder Research Institute(IGFRI), Jhansi as well as National Research Centre for Agro-forestry (NRCAF), Jhansi during September to December, 1994 and 1995. Dispersal units were cleaned, dried in sun and stored in Polythene bags at room temperature.

The diaspores(dispersal units) of <u>Bothriochloa</u> <u>intermedia</u> and <u>Dichanthium</u> <u>annulatum</u> grasses were a 'diad' while the diaspore of <u>Chrysopogon fulvus</u> was a 'triad' and the diaspore of <u>Pennesitum</u> <u>pedicellatum</u> was a 'bur' and the diaspore of <u>Panicum</u> <u>maximum</u> was a single unit.

- 1. The diaspore of 'diad' consisting of 2 spikelets, one sessile and fertile (hermaphrodite) possessing the grain and the other pedicelate and sterile (staminate) e. g.B. intermedia and D. annulatum (Plate 6).
- 2. <u>C</u>. <u>fulvus</u> consisting of three spikelets one sessile and fertile (hermaphrodite) possessing grain, while the other two are pedicelate and sterile (staminate).
- 3. In P. pedicellatum the diaspore unit is a bur (in volucellate spikelet).
- 4. In P. maximum the diaspore is a single unit.

In the present programme two kinds of germination studies have been taken (i) with a single sessile spikelet in all the species except in P. pedicellatum where the bur was used and which will be referred as spikelets throughout these studies (ii) with seeds (caryopsis) obtained by removing the husk (glumes, lemma, palea etc.) mechanically, hence referred

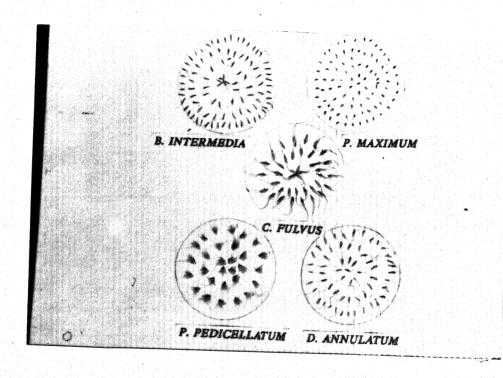


PLATE 6: DISPERSAL UNITS (SPIKELETS) OF FIVE RANGE GRASSES

to as seed. Thus in the case of  $\underline{B}$ . intermedia,  $\underline{D}$ . annulatum,  $\underline{C}$ . fulvus and  $\underline{P}$ . maximum, the germination units taken during the studies were the sessile spikelets or seeds (caryopsis). While in the case of  $\underline{P}$ . pedicellatum the germination unit used was an intact spikelet (bur) or seed. Therefore, the term diaspore has been used for the whole dispersal units in this investigation.

The details of all the specific treatments are described for each experiment, but standard conditions were used for all germination tests.

The germination studies were conducted in petridishes on whatman germination test papers soaked double distilled water. The seeds/spikelets were kept in between two germination test papers replicated five times with 100 spikelets/seeds. These petridishes were then placed in germination cabinets of the seed germinator at 32± 1° C.

## **DORMANCY STUDIES:**

Tests for seed germinability of freshly collected seeds (obtained from the freshly collected spikelets by dehusking) of all the five grasses were conducted within one month from the date of initial seed collection to ascertain, if the seeds required any dormant period or not.

## **SCARIFICATION STUDIES:**

Seven treatments were imposed on fresh (upto one month old seeds) and nine months old spikelets. The treatments were: T1- control (no treatment); T2- Pre-chilling i.e. spikelets were moistened in petridishes and kept in refrigerator for 7 days at 5° C before germination test; T3- hot water: spikelets emerged in hot water at 70° C for 10 minutes before the germination test; T4-heat treatment: spikelets placed in oven at 60° C for 24

hours; T-5 ethanol: spikelets soaked in 95% ethanol for 10 minutes and subsequently washed before germination test; T6- potassium nitrate: spikelets moistened with 500 ppm solution of KNO<sub>3</sub> at the initiation of germination and subsequently with distilled water; T7-gibberellic acid: spikelets moistened with 500 ppm of GA<sub>3</sub> at the start of experiment and thereafter with distilled water. Germination counts were made at regular intervals and upto 14th days.

## **EFFECT OF STORAGE ON SEED GERMINATION OF RANGE GRASSES:**

Test for seed germinability and viability were conducted at three monthly intervals upto 24 months for spikelets/seeds(seeds obtained by dehusking of spikelets at three months intervals).

# RATE OF GERMINATION:

Nine months old spikelets/seeds (obtained by dehusking of 9 months old spikelets) were used to compare the rate of germination between spikelets and seeds.

# ISOLATION AND CHARACTERISATION OF GERMINATION INHIBITORS:

For the purpose of isolation and characterisation of germination inhibitors, diaspores of grasses were used. The isolation and characterisation was conducted by extracting the compounds with suitable solvents followed by paper chromatography and colour reactions following Ibrahim and Towers (1960), Hais and Mecek (1963), Seikel (1964), Harborne (1967, 1973 a) and Markham (1982).

Preliminary investigations for the characteristics test for detection of phenolic compounds in the diaspores were conducted by testing the diaspores leachate (i.e. 10 gm of diaspores soaked in 200 ml of methanol for 24 hrs.) with phenolic reagents viz., alcoholic

ferric chloride (1 g of FeCl<sub>3</sub> dissolved in 100 ml of 95% ethanol), and diazotised sulphanilic acid (Table 1). According to Seikel (1964) 2-3 ml of methanollic extract was taken in a test tube and few drops of FeCl<sub>3</sub> were added. Production of blue or brown colour was noted as indication of presence of phenolic compounds.

Characteristic tests for the detection of phenolic compounds in methanolic extract by phenolic reagents revealed the presence of phenolic onium-ion (anthocyanins) in the diaspores of <u>B</u>. <u>intermedia</u> and <u>P</u>. <u>pedicellatum</u>. In case of <u>D</u>. <u>annulatum</u>, <u>C</u>. <u>fulvus</u> and <u>P</u>. <u>maximum</u> no anthocyanin pigmentation was observed and diaspores leachate gave a positive test for phenolics. Therefore, the following technique was adopted for further studies.

Table 1: Sprays Used for Detecting Phenolic Compound on Paper Chromatogram (PC)

Sprays	Composition
1.Diazotised sulphanillic acid	A 0.3% solution of sulphanillic acid in 8% HCl (25 ml) is mixed with 5% sodium nitrate solution (1.5 ml) just prior to use. The PC is sprayed with this and then with a 20% solution of sodium carbonate before drying. Most compounds with free phenolic hydroxyl group show as yellow, orange, or red to brown spots.
2. Diazotised p- nitroaniline	25 ml of solution of p-nitroaniline (0.3%) in 80%. HCl is mixed with 1.5 ml of 5% sodium nitrite solution just before spraying. The PC is sprayed with this and then with a 20% solution of sodium carbonate before drying. Most of the compounds shows as purple, pink or brown spots.
3. FeCl <sub>3</sub> .K <sub>4</sub> Fe(CN) <sub>6</sub>	1% alcoholic solution of both the compounds is mixed in equal volume just prior to use. Most of the phenolic compounds show blue spots.

Ref: Seikel, 1964; Merkham; 1982.

# **ISOLATION TECHNIQUE:**

ISOLATION OF ANTHOCYANIN FROM B. INTERMEDIA AND P. PEDICELLATUM DIASPORE:

Weighed quantity of diaspores (250 g) of <u>B</u>. intermedia (about 2-3 months of age) were first extracted (in soxhlet extractors) with non polar organic solvents such as petroleum ether and chloroform to remove non-phenolic substances viz., chlorophyll, waxes, fats, water soluble salts etc. (Seikel, 1964). Thereafter, the diaspores were extracted with methanolic HCI (methanol-HCI, 97:3 v/v). The methanolic extract was then filtered through whatman No. 1 filter paper and concentrated by distilling method on a water bath. The residue was now applied on a folded whatman No. 3 mm. Chromatographic paper, 8 cm from the side edge and 3 cm in from the last fold

Table 2: Solvent Used for Paper Chromatographic Analysis of Phenolics

Solvents	Solvents Composition	Approximate running time
BAW	n-butanol:acetic acid: water (4:1:5) mixed thoroughly in a separating funnel), upper phase used	12 to 15 hrs
IBW	isopropanol:butanol:water (140:20:60)	-do-
Forestal	acetic acid:water:HCl (30:10:3)	6 to 8 hrs
Formic	formic acid : water: HCl (5:3:2)	-do-
*15% HOAC	15% acetic acid	4 to 6 hrs
1% HCI	water:conc. HCl (97:3)	5 to 6 hrs
HOAC- HCI	acetic acid:HCl:water (15:3:82)	6 to 8 hrs.

Ref: Seikel, 1964; Harborne, 1967, 73 a; Markham, 1982.

(Fig.1) and allowed to run in BAW (for solvent composition, see Table 2) in a chromatocab (Plate 7) by descending chromatography (Markham, 1982). The anthocyanin appeared as a clear discrete coloured band, which were then cut from the dried paper and the pigment was eluted with methanolic acetic acid(methanol containing 1.0% acetic acid). The elutes were collected and concentrated by repeating the process according to Harborne (1967 and 1973 a). The chromatographically purified pigments was used for the determination of Rf. values in different solvents by descending chromatography on whatman No.1 chromatographic paper. Solvents used for anthocyanin pigment (glycoside) were BAW and 1% HCI (Table 1). A part of the chromatographically purified pigment was acid hydrolyed with 2 NHCI for ½ hrs in a water bath and the anthocyanidin (aglycone) was collected in amylalcohol. Finally the identity of cyanidin (aglycone) was confirmed by cochromatography with the authentic sample in Forestal, Formic and BAW.

In case of <u>P</u>. <u>pedicellatum</u> freshly collected diaspores (reddish purple in colour) were used for the extraction of anthocyanin pigment and the procedures given above for <u>B</u>. <u>intermedia</u> was also followed in this case for isolation of phenolic onium-ion.

ISOLATION OF PHENOLIC COMPOUNDS FROM C. FULVUS, P. PEDICELLATUM, D. ANNULATUM, B. INTERMEDIA AND P. MAXIMUM:

500 g of 10-15 months old diaspores were first extracted with petroleum ether in soxhlet extractor to remove non poloar and non phenolic substances viz., chlorophyll, waxes, fats, water soluble salts(Seikel, 1964). Thereafter, the used material was again extracted in hot MeOH: H<sub>2</sub>O (9:1). The extract was concentrated to 50-60 ml by <u>in vaccuo</u> distillation. The resultant aqueous extract was again extracted (in a separating funnel) with chloroform and the process repeated several times. The purified aqueous extract was now

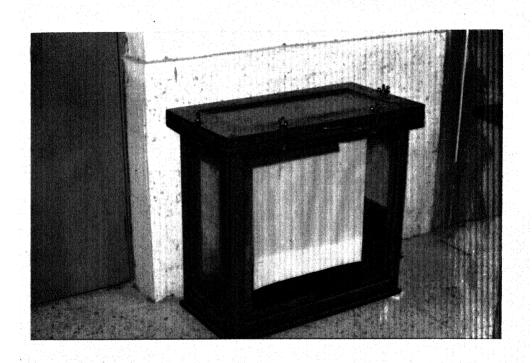


PLATE 7: A CHROMATOGRAPHY CHAMBER

nydrolysed with 2N HCl on a water bath and filtered through whatman No. 1 filter paper. The filtrate was then extracted for ether and ethyl acetate soluble phenolic substances with the help of seperating funnel. The ether and ethyl acetate soluble fractions were dissolved into 5% sodium carbonate solution, acidified to pH 2 and again extracted with ether and ethyl acetate, respectively (Ibrahim and Towers, 1960). Both the extracts were evaporated to near dryness and the residues were taken into a small amount of ethanol and combined.

The combined residue was now applied evenly to a whatman No. 3 chromatography paper, 3 mm diameter at a point about 8 cm from the side edge and 3 cm from the last fold which was folded as illustrated in Fig. 1 to permit the securing of the paper in a through for descending chromatography. The spotted paper was allowed to run in BAW for 15 hrs approximately in a chromatocab. The chromatogram was then trimmed off and refolded for descending chromatography in second dimension using 15% acetic acid for 5 to 6 hrs. The chromatogram was then viewed under ultravoilet light for spot detection with and without ammonia vapours. All the visible spots were pencilled in and eluated in 95% ethanol. Ethanol was evaporated and the spot residues were applied on whatman No.1 chromatographic paper (No. 1 chromatography). The chromatograms were developed by descending chromatography in one dimension using BAW, and 15% acetic acid. Rf. (X100) of the spots were determined by colour reactions with diazotised sulphanillic acid, pnitroaniline and FeCl<sub>3</sub>. K<sub>4</sub> Fe(CN)<sub>6</sub> (Table 1). Finally the identity of the same compounds were confirmed by co-chromatography with the authentic samples (since identity of the remaining phenolics could not be confirmed as the standard samples of many of others were not available).

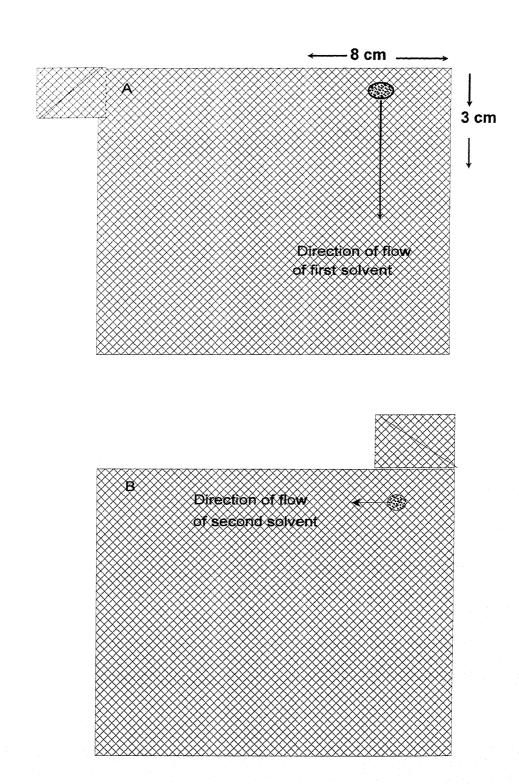


Fig 1 (A-B). Paper Chromatography Methodology.

### **BIOASSAY STUDIES:**

The most widely used bioassy for allelopathic activity is the study of inhibition of seed germination and root as well as shoot growth. Therefore, bioassy studies were conducted to know the effect of water extract of the diaspore, on seed germination as well as root and shoot growth with the respective grass species and with two test species viz., radish (Raphanus sativus) and black gram (Vigna radiatus). Leachate obtained from all the respective grasses was diluted as 25, 50 and 100% concentration and one treatment was also kept as control (distilled water). Radish seeds have been widely used for bioassay studies owing to high and uniform germination and while black gram was also selected due to its rapidity of germination. Seeds of raddish cv. Japanese white were obtained from commercial source while seeds of black gram were collected from farm of NRCAF, Jhansi.

The visible colours of leachate of <u>C</u>. <u>fulvus</u>, <u>P</u>. <u>pedicellatum</u>, <u>D</u>. <u>annulatum</u>, <u>B</u>. <u>intermedia</u> and <u>P</u>. <u>maximum</u> was light yellow, brown, yellow, dark brown and light brown, respectively (Plate 8).

The percentage inhibition of seed germination, root and shoot growth were computed as follows:

Percent(%) inhibition = 100x (N-n/N); where 'N' is the (%) germination or root growth or shoot growth in control and 'n' is the germination percent or root growth or shoot growth in the treatments.

### STATISTICAL ANALYSIS

All the data recorded were put to statistical analysis by converting the percentage data in angular values (Panse and Sukhatme, 1967).

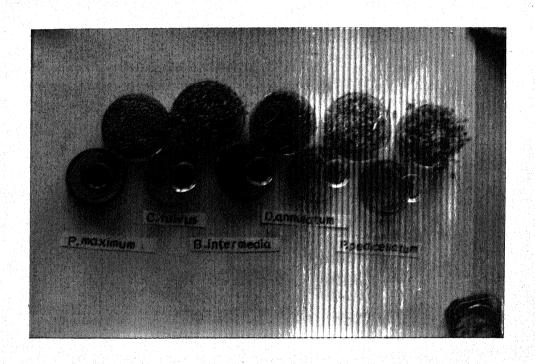


PLATE : AQUEOUS EXTRACTS OF DIASPORE OF FIVE RANGE GRASSES

# RESULTS

# **RESULTS**

# Test Weight of Range Grasses:

Perusal of data in Table 3 revealed that the test weight of spikelets, seeds and husks was significantly differed in these grasses (Appendix 1). The maximum test weight of spikelets (3.31 g), seeds (0.95 g) and husks (2.36 g) was recorded with  $\underline{C}$ . fulvus which was significantly higher than other four grasses. The minimum test weight of spikelets (0.72 g) and husk (0.21 g) was noted with  $\underline{P}$ . pedicellatum while the minimum test weight of seeds (0.30 g) was recorded with  $\underline{B}$ . intermedia. Thus, it was observed that the heaviest seeds/spikelets were with  $\underline{C}$ . fulvus followed by  $\underline{P}$ . maximum (Table 3).

### **Dormancy Studies:**

Dormancy studies conducted as freshly (within month) collected seeds of five range grasses revealed that spikelets of three grasses namely Pennisetum pedicellatum, Bothriochloa intermedia and Panicum maximum did not germinate while Chrysopogon fulvus and Dichanthium annulatum showed germination which was at par and very low (3.6%). Germination of dehulled seeds also showed the similar trend except in case of B. intermedia which exhibited 12.4% germination. Germination of dehulled seeds of D. annulatum and C. fulvus showed about 12 and 8 times higher than the germination of spikelets tested at the same period (Table 4).

Table 3: Test Weight of Range Grasses (Weight of 1000 Seeds)

Grasses	Weight of Spikelets(g)	Weight of Seeds (Caryopsis) (g)	Weight of Husks (g)
Chrysopogon fulvus	3.31	0.95	2.36
Pennisetum pedicellatum	0.72	0.51	0.21
Dichanthium annulatum	0.96	0.36	0.60
Bothriochloa intermedia	0.79	0.30	0.49
Panicum maximum	1.14	0.82	0.32
SEm ±	0.05	0.05	0.06
CD 5%	0.15	0.15	0.18
Mean	1.34	0.58	0.78

Table 4: Germination of Freshly Collected Spikelets and Seeds (%)

Grass Species	Germination (%)						
	Spikelets	S.D. <u>+</u>	Seeds	S.D. <u>+</u>			
Chrysopogon fulvus	3.60	1.49	27.20	2.03			
Pennisetum pedicellatum	0	0	0	0			
Dichanthium annulatum	3.60	1.49	42.40	6.74			
Bothriochloa intermedia	0	0	12.40	5.71			
Panicum maximum	0	0	0	0			

# Effect of Scarification and Chemical Treatments on Germination of Spikelets:

## Chrysopogon fulvus

Germination studies conducted on fresh collected spikelets (within month) revealed that there was significant increase in seed germination of this species due to heat

treatment and gibberellic acid (Appendix II). The germination recorded with pre-chilling, hot water and potassium nitrate treatments was higher than control. However, the increase was statistically not significant (Table 5a, Fig.-2A). Thus, the results showed that due to heat treatment and application of GA<sub>3</sub>, the dormancy of C. fulvus may be reduced. The highest germination of 26.0% was noted with GA<sub>3</sub> followed by heat treatment (24.6%) while minimum germination of 3.6% was recorded in control. There was no germination with ethanol treatment.

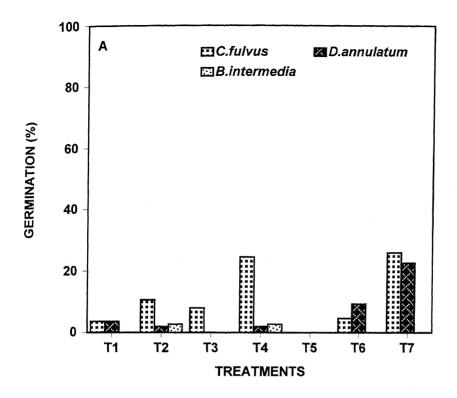
Germination study conducted at nine months stored spikelets revealed that maximum germination was recorded with KNO<sub>3</sub> (56.0%) followed by control (53.3%). The minimum germination of 36.0% was noted with pre-chilling treatment which was significantly lower than other treatments (Table 5b, Fig. 2B, Appendix III). There was no germination with ethanol treatment.

### Pennisetum pedicellatum

There was no germination of freshly collected spikelets of P. pedicellatum. However, in case of storage for 9 months spikelets showed significant differences in germination (Appendix III). The maximum germination of 86.6% was recorded with treatment consisting of KNO<sub>3</sub> followed by hot water treatment (78.6%) and heat treatment (76.0%). Germination recorded with control and GA<sub>3</sub> was at par (70.6%). The minimum germination was observed with pre-chilling treatment (Table 5b, Fig.2B). There was no germination with ethanol treatment.

### Dichanthium annulatum

Data presented in Table 5a (Fig.2A) revealed that there was no dormancy in the spikelets of <u>D</u>. <u>annulatum</u> as 3.6% germination was recorded in freshly collected spikelets. However, germination can be significantly increased with application of GA<sub>3</sub> i.e. 22.7%



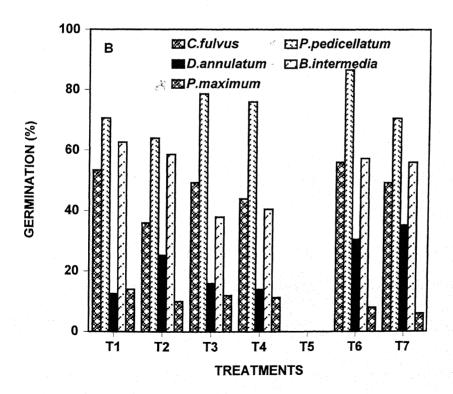


Fig.2 (A-B). Effect of scarification and chemical treatments on germination (%) of freshly collected (A) and nine months storage (B) of

(Appendix II). There was no effect of hot water treatment in the germination

Table 5a: Effect of Scarification and Chemical Treatments o Germination (%) of Spikelets (freshly collected)

Treatments	C.	Р.	D.	В.	P.
	fulvus	pedicellatum	annulatum	intermedia	maximum
Control	3.6 (11.28)	O O	3.6 (11.28)	0	0
Per-chilling	10.6 (19.05)	0	2.0 (8.13)	2.7 (5.47)	0
Hot-Water	8.0 (15.05)	0			0
Heat	24.6 (29.45)	0	2.0 (8.13)	2.7 (5.47)	0
Ethanol	0	0	0	0	0
Potassium Nitrate, 500 ppm	4.6 (12.42)	. 0	9.3 (17.52)	0	0
GA <sub>3</sub> , 500 ppm	26.0 (30.46)	0	22.7 (32.77)	0	0
SEm <u>+</u>	3.13	0	2.11	0	0
CD 5%	9.66	0	6.50	0	0

Values in parentheses are angular values

of this grass. There was no germination with ethanol treatment.

In case of nine months storage, spikelets showed significant increase in germination in all the treatments (Appendix III). The germination recorded with application of GA<sub>3</sub>, KNO<sub>3</sub> and pre-chilling treatment showed significantly higher germination with control as well as hot water and heat treatments (Table 5b, Fig.2B). There was no germination with ethanol treatment.

### Bothriochloa intermedia

Perusal of table 5a showed that there was no germination of freshly collected

spikelets of <u>B</u>. <u>intermedia</u> in control as well as other treatments except pre-chilling and heat treatments exhibited 2.7% germination. This showed that there was a dormancy in spikelets of this grass.

In case of nine months storage of spikelets of this grass showed significant variation in germination (Appendix III). The maximum germination of 62.6% was recorded with control. However, germination recorded with pre-chilling, KNO<sub>3</sub>, GA<sub>3</sub> and control was statistically not differed (Table 5b, Fig.2B). There was no germination with ethanol treatment.

Table 5b: Effect of Scarification and Chemical Treatments on Germination (%) of Spikelets (at nine months storage)

Treatments	C.	P.	D.	B.	P.
	fulvus	pedicellatum	annulatum	intermedia	maximum
Control	53.3	70.6	12.6	62.6	14.0
	(46.1)	(57.42)	(20.60)	(52.34)	(21.75)
Pre-Chilling	36.0	64.0	25.3	58.6	10.0
	(36.8)	(53.19)	(30.17)	(49.88)	(18.06)
Hot Water	49.3	78.6	16.0	38.0	12.0
	(44.6)	(63.26)	(23.47)	(38.02)	(20.09)
Heat	44.0	76.0	14.0	40.6	11.3
	(41.5)	(60.88)	(21.83)	(39.60)	(19.32)
Ethanol	0	0	0	0	0
Potassium	56.0	86.6	30.6	57.3	8.0
Nitrate, 500 ppm	(48.5)	(69.44)	(33.55)	(49.26)	(16.35)
GA <sub>3</sub> , 500 ppm	49.3	70.6	35.3	56.0	6.0
	(44.6)	(57.25)	(39.40)	(48.49)	(14.05)
SEm <u>+</u>	2.17	3.68	1.80	2.45	3.06
CD 5%	6.70	11.35	5.55	7.57	9.44

Values in Parentheses are angular values

### Panicum maximum

Freshly collected spikelets of P. maximum showed dormancy as there was no

germination (Table 5a) in any treatment.

Germination of spikelets at 9 months storage exhibited significant variations (Appendix III) This showed maximum germination of 14.0% which was recorded with control followed by hot water treatment (12.0%) and heat treatment (11.3%). The germination recorded with application of  $GA_3$  was minimum (Table 5b, Fig. 2B). The results showed that there was no effect of mechanical as well as chemical treatments on germination of F. maximum. Although there was no germination with ethanol treatment.

Thus the results showed that three grasses viz.  $\underline{P}$ .  $\underline{pedicellatum}$ ,  $\underline{B}$ .  $\underline{intermedia}$  and  $\underline{P}$ .  $\underline{maximum}$  showed dormancy as there was no germination in freshly collected spikelets while two grasses ( $\underline{C}$ .  $\underline{fulvus}$  and  $\underline{D}$ .  $\underline{annulatum}$ ) showed that there was no dormancy as there was germination in freshly collected seed. Further it was observed that application of  $GA_3$  enhanced the germination in  $\underline{C}$ .  $\underline{fulvus}$  and  $\underline{D}$ .  $\underline{annulatum}$  as compared to other mechanical and chemical treatments in freshly collected spikelets.

In case of nine months collected spikelets there was a significant differences in germination due to different treatments in all the five grass species. However, the highest germination was recorded with control in 3 grasses (C. fulvus, B. intermedia and P. maximum. This showed that there was no effect of either mechanical or chemical treatments on germination of these grasses. But in two grasses viz., P. pedicellatum and D. annulatum the maximum germination was recorded with application of KNO<sub>3</sub>, GA<sub>3</sub>, respectively. This showed that these two grasses required chemical treatments for higher germination. (Table 5b. Fig. 2B).



# Effect of Storage (age) and Removal of Glumes on Germination:

Germinability of spikelets and seeds (obtained by dehusking of spikelets) at three month intervals:

Germination percentage of stored spikelets started increasing with the increase in period of storage. After three months of storage, loss in dormancy started and therefore, an increasing trend in germination was observed. The increasing trend in germination was maintained up to 9 and 12 months of storage and thereafter reduction in percentage germination was recorded. The detail of all the five grass species are given as below:

### Chrysopogon fulvus

The spikelet of this grass showed germination in freshly collected seeds, (i.e. within a month of collection, Table 4). The increasing trend in germination of spikelet was observed upto 9 months (53.6%). After that a decrease in germination was observed. Although there was no significant differences in between germination of 9 and 12 months of storage (Table 6a, Fig. 3A).

Similarly significant increase in germination was observed due to storage of seeds (Appendix IV). The maximum germination of seeds was observed at 18 months of storage. However, there was no significant differences in germination of seeds stored for 9th, 12th, 15th and 18th months (Table 6b, Fig. 3B).

### Pennisetum pedicellatum

In this grass species, dormancy was observed in freshly collected spikelets, but germination started at three months storage. The maximum germination of spikelets (70.4%) was recorded at 9 months of storage (Table 6a, Fig. 3A) which was significantly higher than other storage periods except 12 and 15 months of storage (Appendix IV). The

significantly lower germination was observed at 24 months of storage.

Table 6a: Effect of Storage on Spikelets and its Germination (%)

Age in Months	C. fulvus	P. pedicellatum	D. annulatu m	B. intermedi a	P. maximum	SEm ±	C.D.5%
3	21.2 (28.5)	32.0 (34.5)	7.6 (15.5)	4.6 (12.0)	-	1.74	5.24
6	39.6 (39.2)	40.8 (39.1)	31.2 (33.7)	71.2 (57.9)	28.4 (31.7)	4.20	12.61
9	53.6 (47.0)	70.4 (57.2)	42.4 (40.6)	72.8 (58.7)	64.0 (53.2)	1.66	4.99
12	52.0 (46.2)	65.6 (54.1)	57.6 (49.4)	62.4 (52.2)	59.2 (50.3)	1.03	3.09
15	48.8 (44.4)	64.8 (53.6)	48.0 (43.4)	60.0 (50.4)	32.8 (34.9)	1.43	4.29
18	44.0 (41.6)	60.8 (51.3)	36.8 (37.3)	49.6 (43.9)	21.6 (27.6)	3.44	10.32
21	31.2 (33.9)	25.6 (30.3)	33.6 (35.4)	40.0 (39.2)	19.4 (25.8)	1.40	4.19
24	24.0 (29.2)	8.8 (16.7)	32.0 (34.5)	32.4 (34.7)	18.6 (25.3)	1.74	5.22
SEm <u>+</u>	1.36	2.70	1.95	2.16	2.19		
CD 5%	3.94	7.82	5.65	6.25	6.35		

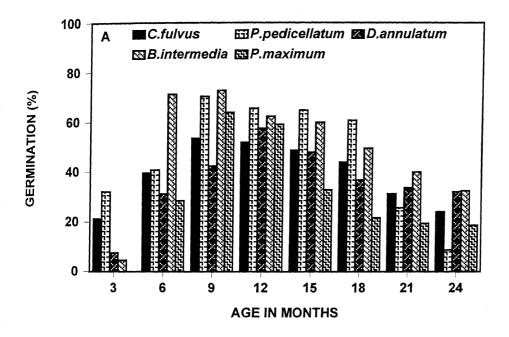
Values in parentheses are angular values

As regards to germination in seeds of <u>P</u>. <u>pedicellatum</u> showed the maximum and significantly higher germination (85.6%) at 9 month of storage as compared to other periods of storage except 12 month of storage (Table 6b, Fig. 3B).

### Dichanthium annulatum

In <u>D</u>. <u>annulatum</u> germination started in freshly collected spikelets. An increasing trend in germination with a maximum of 57.6% was noted upto 12 months (Table 6 a, Fig.3A). This value was significantly higher than other storage periods (Appendix IV). After that a decrease in germination was observed.

Similarly in case of seeds of this grass, maximum germination was recorded at 12



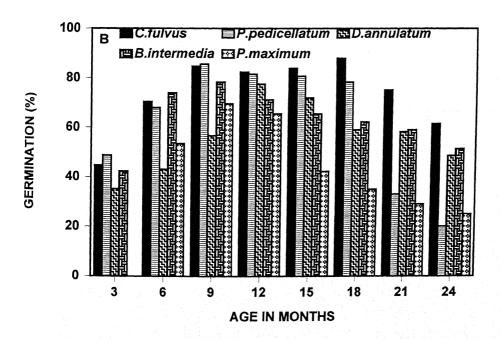


Fig. 3(A- B). Effect of storage of spikelets (A) and removal of glumes (seeds,B) on germination percentage of range grasses.

month of storage (77.6%) which was significantly higher than other storage period except 15 months of storage (Appendix IV, Table 6b, Fig. 3B).

### Bothriochloa intermedia

In <u>B</u>. <u>intermedia</u> dormancy was observed in freshly collected spikelets. But germination started at three months of storage. The maximum germination of 72.8% of spikelets observed at 9 months of storage was significantly higher than other storage periods except in 6 months of storage (Table 6a, Fig. 3A). The significantly lower germination was noted at 3 months of storage.

In case of seeds of <u>B</u>. <u>intermedia</u> it was observed that even the freshly collected seeds exhibited germination (12.4%). The seeds stored for 9 months of period-showed maximum germination of 78.4% which was significantly higher (Appendix IV, Table 6b, Fig. 3B) than other storage periods except at 6 months of storage.

### Panicum maximum

Perusal of data in table 6a,b revealed that the spikelets and seeds of <u>P</u>. <u>maximum</u> showed dormancy even upto 3 months of storage. However, the germination started at 6 months of storage both in spikelets and seeds of this grass.

Table 6b: Effect of Storage of Spikelets and Removal of Glumes (seeds) on Germination

Age in Months	C. fulvus	P. pedicellatum	D. annuiatum	B. intermedia	P. maximum	SEm ±	CD 5%
3 .	44.8 (42.0)	48.8 (44.3)	35.2 (36.1)	42.4 (40.6)	-	2.30	6.91
6	70.4 (56.9)	68.0 (55.5)	43.2 (41.0)	74.0 (59.3)	53.6 (47.1)	1.51	4.53
9	84.8 (67.4)	85.6 (67.9)	56.8 (48.9)	78.4 (62.5)	69.6 (56.6)	1.62	4.87
12	82.4 (65.3)	81.6 (64.7)	77.6 (61.9)	71.2 (57.6)	65.6 (54.1)	1.43	4.30
15	84.0 (66.8)	80.8 (65.9)	72.0 (58.2)	65.6 (54.1)	42.4 (40.6)	1.83	5.51
18	88.0 (70.5)	78.4 (62.4)	59.2 (50.3)	62.4 (52.2)	35.2 (36.1)	2.30	6.90
21	75.2 (60.3)	33.2 (34.9)	58.4 (49.9)	59.2 (50.4)	29.2 (32.7)	1.82	5.46
24	61.6 (51.8)	20.2 (26.4)	48.8 (44.3)	51.6 (45.9)	25.2 (29.9)	3.55	10.65
் SEm <u>+</u>	1.99	1.59	2.02	1.60	1.87		
CD 5%	5.76	4.61	5.85	4.64	5.44		

Values in parentheses are angular values

The maximum germination of 64.0% and 69.6% was recorded in spikelets and seeds, respectively at nine months of storage which was significantly higher than other periods of storage (Appendix IV)except 12 months (Table 6a,b, Fig.3A,B).

# Comparison of Germination of Different Grasses at Different Storage Period

From perusal of Table 6a and 6b it is clear that the variation in germination of spikelets as well as seeds of different grasses was found significant at all the storage periods (from 3 to 24 months, Appendix IV). During 3 months of storage the maximum germination in spikelets as well as seeds was observed in P. pedicellatum followed by C. fulvus while in case of P. maximum germination was not observed. At six months of

storage the maximum germination in spikelets as well as seeds was noted in <u>B</u>. <u>intermedia</u> which were significantly higher than other grass species except with <u>C</u>. <u>fulvus</u> for seed.

At nine months of storage maximum germination in spikelets and seeds was recorded in  $\underline{B}$ . intermedia (72.8%) and  $\underline{P}$ . pedicellatum (85.6%), respectively. However, there was no significant differences in the germination of  $\underline{B}$ . intermedia and  $\underline{P}$ . pedicellatum for spikelets and  $\underline{P}$ . pedicellatum and  $\underline{C}$ . fulvus for seeds.

At 12 months of storage germination in spikelets of <u>P</u>. <u>pedicellatum</u> was significantly higher as compared to other grass species except <u>B</u>. <u>intermedia</u> while in case of seeds significantly higher germination was recorded with <u>C</u>. <u>fulvus</u> compared to other grasses except <u>P</u>. <u>pedicellatum</u>.

At 15 months of storage germination in spikelets of <u>P</u>. <u>pedicellatum</u> was significantly higher as compared to other grass species except <u>B</u>. <u>intermedia</u> while in case of seeds significantly higher germination was recorded with <u>C</u>. <u>fulvus</u> as compared to other grasses except <u>P</u>. <u>pedicellatum</u>.

At 18 months of storage the maximum and significantly higher germination in spikelets as well as seeds was recorded in <u>P. pedicellatum</u> (60.8%) and in <u>C. fulvus</u> (88.0%), respectively.

At 21 months of storage the significantly higher germination was observed in <u>B</u>. <u>intermedia</u> (40.0%) in case of spikelets and <u>C</u>. <u>fulvus</u> (75.2%) in case of seeds and the minimum germination was recorded in <u>P</u>. <u>maximum</u> both in spikelets and seeds.

At 24 months of storage maximum germination in spikelets was recorded in <u>B</u>. intermedia (32.4%) and <u>C</u>. fulvus (61.6%) in case of seeds. However, there was no significantly differences in the germination of <u>B</u>. intermedia and <u>D</u>. annulatum for spikelets and <u>C</u>. fulvus and <u>B</u>. intermedia for seeds (Table 6a,b).

# Rate of Germination of Spikelets and Seeds in Range Grasses

Nine month old spikelets as well as seeds (seeds obtained by dehusking of 9 months stored spikelets) were used for the studies on rate of germination. Data presented in Table 7 (Fig.4A,B,C, D & E) showed that germination of spikelets started within 24hr in C. fulvus, P. pedicellatum and B. intermedia. While in case of D. annulatum germination started after 24hr and in P. maximum it was started after 48 hr.

In case of seeds of all the grasses the germination started within 24 hr and it was maximum of 46.7% in case of <u>C</u>. <u>fulvus</u> and minimum of 3.2% in case of <u>P</u>. <u>maximum</u>. Chrysopogon fulvus:

Germination of spikelets started within 24 hr of sowing in petridishes and percentage germination increased from 10.7% to 45.3% on 4th day after sowing. After 4th days of sowing percentage germination increased from 45.3% to 50.7% on the 5th days (Table 7, Fig.4A). No germination was observed after 5th day onwards.

In case of seeds, rate of germination was comparatively higher and 46.7% germination was observed after 24 hr of sowing (Table 7, Fig.4A). Most of the seeds exhibited germination between 2nd and 3rd day after sowing. The percentage germination increased from 72.0% on 2nd day to 86.7% on 4th day. No seed germination was recorded after 4th day onwards.

# Pennisetum pedicellatum:

Germination of spikelets started within 24 hr of sowing in petridishes and percentage germination increased from 7.2% to 41.6% on 2nd day after sowing. After 3rd day of sowing percentage germination increased from 65.6% to 70.4% on the 4th day (Table 7, Fig. 4B). No germination was observed after 4th day onwards.

In case of seeds, germination started within 24 hr of sowing (1st day) and continued up to 4th day. Percentage germination increased from 49.6% on 1st day to 83.2% on 3rd day. The increase in percentage germination from 83.2% on 3rd day to 85.6% on 4th day (Table 7, Fig. 4B). No germination was observed after 4th day onwards.

### Dichanthium annulatum:

Germination of spikelets started from 2nd day onwards and continued upto 11th day after sowing. Percentage germination increased from 8.8% on 2nd day to 20.8% on 5th day while from 6th to 11th day the increase in percentage germination was high (20.8% to 42.2%) (Table 7, Fig. 4C).

In case of seeds, germination started within 24 hr of sowing (1st day) and continued upto 11th day. Percentage germination increased from 5.6% on 1st day to 27.2% on 4th day. The increase in percentage germination was observed from 27.2% on 4th day to 56.8% on 11th day (Table 7, Fig. 4C).

#### Bothriochloa intermedia:

Germination of spikelets started within 24hr of sowing in petridishes and percentage germination increased from 16.8% to 55.2% on 3rd day after sowing. After 3rd day of sowing, percentage germination increased from 55.2% to 72.8% on the 6th day (Table 7, Fig. 4D). No germination was observed after 6th day onwards.

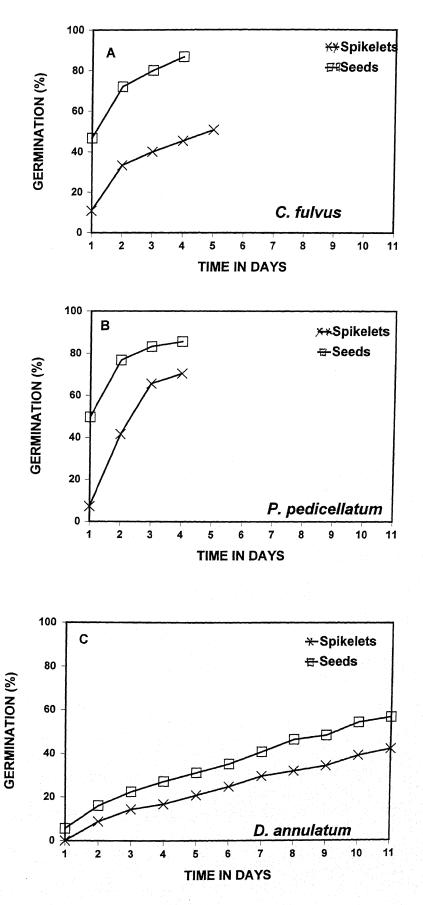


Fig. 4 (A-C). Rate of germination of spikelets and seeds of range grasses.

Table 7: Rate of Germination of Spikelets and Seeds of Range Grasses

	%	% Germination Achieved in Days (mean of five replications)									
J	1	2	3	4	5	6	7	8	9	10	11
Chrysopogon fulvus 1 2	10.7 46.7	33.3 72.0	40.0 80.0	45.3 86.7	50.7	-	-	-	-	-	-
Pennisetum pedicellatum 1 2	7.2 49.6	41.6 76.8	65.6 83.2	70.4 85.6	-	<b>~</b>		-	-	-	-
Dichanthium annulatum 1 2	- 5.6	8.8 16.0	14.4 22.4	16.8 27.2	20.8 31.2	24.8 35.2	29.6 40.8	32.0 46.4	34.4 48.4	39.2 54.4	42.2 56.8
Bothriochloa intermedia 1 2	16.8 17.6	42.4 46.4	55.2 64.8	62.4 74.4	68.0 78.4	72.8 -	- - ·	-	-	<u>-</u>	-
Panicum maximum 1 2	- 3.2	- 12.8	7.2 24.2	17.6 35.2	24.8 41.6	32.8 48.0	39.2 52.0	44.8 56.8	52.0 62.4	59.2 67.2	64.0 69.6

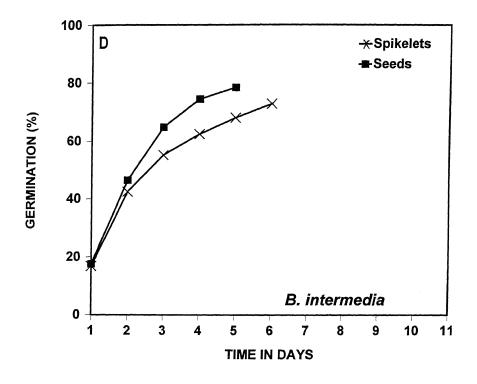
1. Spikelets; 2. Seeds

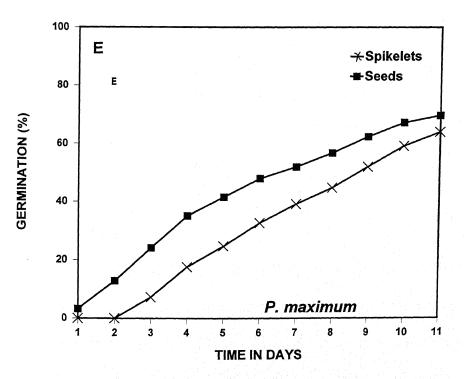
In case of seeds, germination started within 24hr of sowing (1st day) and continued upto 5th day. Percentage germination increased from 17.6% to 64.8% on 3rd day. The increase in percentage germination from 64.8% on 3rd day to 78.4% on 5th day (Table 7, Fig. 4D) was observed. No germination was observed after 5th day onwards.

### Panicum maximum:

Germination of spikelets started from 3rd day onwards and extended up to 11th day after sowing. Percentage germination increased from 7.2% on 3rd day to 32.8% on 6th day. The increase in percentage germination was recorded from 32.8% on 6th day to 64.0% on 11th day (Table 7, Fig. 4E).

In case of seeds, the germination started within 24 hr of sowing (1st day) and continued upto 11th day. Percentage germination increased from 3.2% on 1st day to





(D -E). Rate of germination of spikelets and seeds of range grasses.

41.6% on 5th day to 69.6% on 11th day was noticed (Table 7, Fig. 4E).

# Isolation and Characterisation of Germination Inhibitors

### Pennisetum pedicellatum:

Chromatography of the methanolic HCI extract on whatman 3mm revealed the presence of two magenta spots (A and B). Further co-chromatography of the hydrolysed glycoside (aglycone) with cyanidin chloride indicated the presence of cyanidin (Table 8).

Table 8: Rf. Values of Cyanidin Glycosides (anthocyanin) and Cyanidin (anthocyanidin) Extracted from the Diaspore of P. pedicellatum

Anthocyanin	Rf	x(100) in	Со	lour in
Pigment	BAW	1% Hcl	Visible	U.V.
Cyanidin A	40	05	magenta	dull magenta
Glycosides B	31	27	magenta	dull magenta
			1	
Anthocyanidin	Rf	(x100)in	Col	our in
(aglycone)	Forestal	BAW	visible	U.V.
Cyanidin	48	62	Purple-brown	magenta
Suspected Cyanidin	47	62	Purple-brown	magenta

Solvents - BAW, n-butanol -acetic acid - water (4:1:5); 1% HCI, conc. HCI - water (97:3); Forestal, acetic acid - conc. HCI - water (30:3:10)

#### Bothriochloa intermedia:

Chromatography of the methanolic HCI extract of diaspore on whatman 3mm paper revealed the presence of one magenta spot. Further chromatographic results on whatman No. 1 paper has been given in Table 9. Co-chromatography of the hydrolysed glycoside (aglycone) with authentic sample of cyanidin chloride revealed the presence of cyanidin. Therefore, the pigment present in the seed enclosed by glumes is a cyanidin glycoside.

Table 9: Rf. Values of the Cyanidin Glycoside (anthocyanin) and Cyanidin (anthocyanidin) Extracted from the Diaspore of <u>B</u>. intermedia

Anthocyanin Pigment	Rf	(x100) in	col	colour in						
	BAW	1% HcI	Visible	U.V.						
Cyanidin glycoside from diaspore	35 17		magenta	dull magenta						
Anthocyanidin	Anthocyanidin Rf (x100) in Colour in									
(aglycone)	Forestal	BAW	Visible	U.V.						
Cyanidin	48	62	purple-brown	magenta						
Suspected Cyanidin	48	62	Purple-brown	magenta						

Solvents are: BAW, n-butanol - acetic acid - water (4:1:5, upper layer); 1% HCl, conc. HCl-water (97:3); Forestal, acetic acid - conc. HCl - water (30:3:10).

# ISOLATION AND CHARACTERISATION OF GERMINATION INHIBITORS Chrysopogon fulvus:

Two dimensional chromatography of hydrolysed methanolic extract of diaspores on whatman 3 mm paper revealed the presence of five major spots. Further co-chromatography with authentic samples on whatman No. 1 followed by colour reactions (in U.V. as well as sprays) indicated the presence of p-hydroxy benzonic acid, vanillic acid, caffeic acid, p-coumaric acid and ferulic acids (Table10).

Table 10: Rf. Values, U.V. Absorption and Colour Reactions of Phenolics Isolated from the Diaspore of <u>C. fulvus</u>

	Rf(x10	Rf(x100) in Flourescence in		Reagent Colour			
	BAW	15 % AA	UV	p-nitroaniline.	Sulphanili c acid	K₄FeCN₅ & (FeCl₃)	
p-hydroxy benzoic acid	91	64	faint	pink		-	
Suspected p- hydroxy benzoic acid	89	62	faint	pink	-	-	
Caffeic acid	78	67	blue abs	bn black	-	blue	
Suspected caffeic acid	79	68	blue abs	bn black	<b>-</b>	blue	
Vanillic acid	84	84		purple	orange	blue	
Suspected vanillic acid	81	82	-	purple	orange	blue	
Ferulic acid	87	72	blue abs	bn black	tan	blue	
Suspected ferulic acid	85	71	blue abs	bn black	tan	blue	
p-coumaric acid	93	68	purple abs	bn black	red	blue	
Suspected p- coumaric acid	92	69	purple abs	bn black	red	blue	

Rf : Relative frequency;

abs : Absorption;

bn: Brown

# Pennisetum Pedicellatum:

Two dimensional chromatography of the hydrolysed methanolic extract of diaspore on whatman 3 mm paper revealed the presence of 5 major spots. Further co-chromatography on whatman No. 1 paper with authentic samples followed by colour reactions (in U.V as well as sprays) indicated presence of p-hydroxy benzoic acid, p-coumaric acid, caffeic acid and ferulic acid (Plate 9, Table 11). Identification of one major



spot could not be done. Ferulic acid gave two spots <u>Cis</u> and <u>Trans</u> isomers in 15% A.A. (Table 11). The <u>trans</u> isomer travels ahead of the <u>Cis</u> isomer.

Table 11: Rf Values, U.V. Absorption and Colour Reactions of Phenolics Isolated from the Diaspore of P. pedicellatum

	Rf(x100) in		Flourescence in	Reagent Colour			
	BAW	15% AA	Ú.V.	p- nitroanilin e	Sulphanili c acid	K₄FeCN <sub>6</sub> &(Fe Cl <sub>3</sub> )	
p-hydroxy benzoic acid	90	64	faint abs	pink	-	-	
Suspected p- hydroxy benzoic acid	89	65	faint abs	pink	-	-	
Caffeic acid	78	67	blue abs	bn black	-	blue	
Suspected caffeic acid	79	66	blue abs	bn black	<b>-</b>	blue	
Ferulic acid	87	72	blue abs	bn black	tan	blue	
Suspected ferulic acid	85	71	blue abs	bn black	tan	blue	
p-coumaric acid	92	68	purple abs	bn black	red	blue	
Suspected p- coumaric acid	91	69	purple abs	bn black	red	blue	

Rf = Relative frequency; abs= Absorption; bn = Brown

### Dichanthium annulatum:

Two dimensional chromatography of hydrolysed methanolic extract of diaspore on whatman 3 mm gave 5 major spots and further co-chromatography on whatman No. 1 paper with authentic samples followed by colour reactions (in U.V. as well as sprays) indicated presence of p-hydroxy benzoic acid, vanillic acid and p-coumaric acid (Table 12). Identification of two major spots could not be done.

Table 12: Rf Values, U.V. Absorption and Colour Reactions of Phenolics Isolated from the Diaspore of <u>D</u>. <u>annulatum</u>

	Rf(x100) in		Fluores- cence in	Reagent Colour		
	BAW	15% AA	υv	p- nitroaniline	Sulfanilic acid	K₄FeCN <sub>6</sub> & (FeCl₃)
p-hydroxy benzoic acid	90	64	faint abs	pink	-	-
Suspected p-hydroxy benzoic acid	91	66	faint abs	pink	-	-
Vanillic acid	84	83	_	purple	orange	blue
Suspected Vanillic acid	83	81	-	purple	orange	blue
p-coumaric acid	92	66	purple abs	bn black	red	blue
Suspected p- coumaric acid	93	67	purple abs	bn black	red	blue

Rf = Relative frequency;

abs= Absorption;

bn = Brown

### Bothriochloa intermedia:

Two dimensional chromatography of hydrolysed methanolic extract of diaspore on whatman 3 mm revealed the presence of 5 major spots. Further co-chromatography on whatman No. 1 paper with authentic samples followed by colour reactions (in U.V. as well as sprays) indicated presence of p-hydroxy benzoic acid, vanillic acid, ferulic acids and p-coumaric acid (Table 13). Identification of one major spot could not be done.

Table 13: Rf. Values, U.V. Absorption and Colour Reactions of Phenolics Isolated from the Diaspore of <u>B</u>. intermedia

	Rf(x100) in		Floures- cence in	Reagent Colour		
	BAW	15% AA	UV	p- nitroaniline	Sulphanilic acid	K₄FeCN <sub>6</sub> & (FeCl₃)
p-hydroxy benzoic acid	90	65	faint abs	pink	_	_
Suspected p-hydroxy benzoic acid	88	64	faint abs	pink	-	-
Vanillic acid	84	83		purple	orange	blue
Suspected vanillic acid	82	81	-	purple	orange	blue
Ferulic acid	87	72	blue abs	bn black	tan ·	blue .
Suspected ferulic acid	87	71	blue abs	bn black	tan	blue
p-coumaric acid	92	67	purple abs	bn black	red	blue
Suspected p- coumaric acid	90	65	purple abs	bn black	red	blue

Rf = Relative frequency;

abs= Absorption;

bn = Brown

#### Panicum maximum:

Two dimensional chromatography of hydrolysed methanolic extract of diaspore on whatman 3 mm gave 5 major spots and further co-chromatography on whatman No. 1 paper with authentic samples followed by colour reactions (in U.V. as well as sprays) indicated presence of p-hydroxy benzoic acid, caffeic acid, ferulic acid and vanillic acid (Table 14). Identification of one major spot could not be done.

Table 14: Rf. Values, U.V. Absorption and Colour Reactions of Phenolics Isolated from the Diaspore of P. maximum

	Rf(x100) in		Floures- cence in	Reagent Colour		
	BAW	15% AA	UV	p- nitroaniline	Sulphanilic acid	K₄FeCN₅ & (FeCl₃)
p-hydroxy benzoic acid	90	64	faint	pink	-	-
Suspected p-hydroxy benzoic acid	90	66	faint	pink	-	-
Caffeic acid	78	67	blue abs	bn black	-	blue
Suspected caffeic acid	78	67	blue abs	bn black	_	blue
Vanillic acid	85	84	_	purple	orange	blue
Suspected vanillic acid	84	82	-	purple	orange	blue
Ferulic acid	86	72	blue abs	bn black	tan	blue
Suspected ferulic acid	85	73	blue abs	bn black	tan	blue

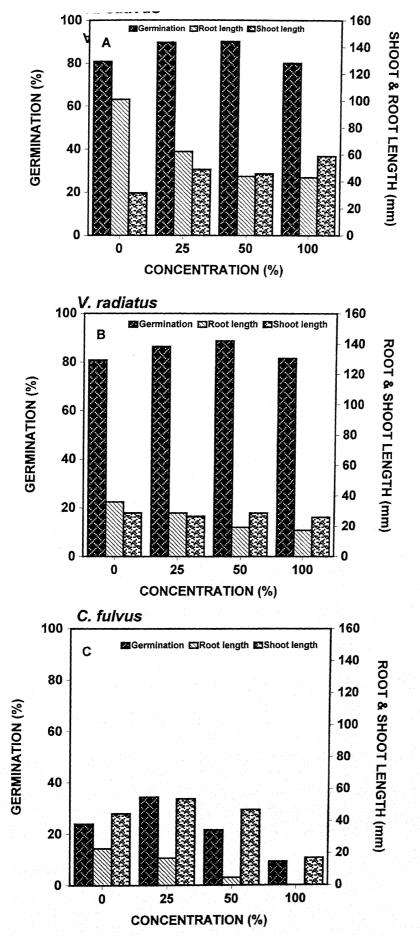
Solvents are: BAW, n-butanol -acetic acid- water (4:1:5 upper layer), 15% AA, 15% Acitic acidRf = Relative frequency; abs= Absorption; bn = Brown

## **Bioassay Studies**

Results of bioassay studies with water extract of spikelets (diaspore) on five range grass species (C. fulvus, P. pedicellatum, D. annulatum, B. intermedia and P. maximum) as well as of two test species namely Raphanus sativus and Vigna radiatus have been presented in table 15 a, b, c to 19 a,b,c. Percentage inhibition of germination and root and shoot growth have been depicted in Fig.5A,B,C to 9A,B,C.

## Chrysopogon fulvus

Bioassay study with the water extract of spikelets of <u>C</u>. <u>fulvus</u> on the germination as well as root and shoot growth of <u>R</u>. <u>sativus</u> has been given in table 15 a (Fig. 5A). It was observed that there was no inhibitory effect of <u>C</u>. <u>fulvus</u> leachate even upto 50%



J. 5(A-C). Effect of C. fulvus diaspore extract on germination and growth root and shoot of *R. sativus* (A), *V. radiatus* (B) and *C. fulvus* (C).

concentration. However, 0.9% inhibition was recorded at 100% concentration. Data on length of root showed that there was significant decrease in root length as the concentration of leachate enhanced from 25 to 100% concentration (Appendix V a). But there was no inhibitory effect of leachate on shoot length of R. sativus even upto 100% concentration. This showed that there was stimulatory effect on shoot length of R. sativus.

Table 15a: Effect of <u>C</u>. <u>fulvus</u> Diaspore Extract on Germination and Growth of Root and Shoot of <u>R</u>. <u>sativus</u>

	CON	CONCENTRATION OF LEACHATE OF C. fulvus						
	Control	25% Conc.	50% Conc.	100% Conc.	SEm±	CD 5%		
Germination (%)	80.8 (64.0)	89.6 (71.2)	90.0 (71.6)	80.0 (63.5)	(0.9)	(2.7)		
Inhibition (%)	_	-10.9	-11.4	0.9				
Root Length (mm)	100.8	62.2	43.8	43.0	4.1	12.6		
Inhibition (%)	• 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	38.3	56.5	57.3				
Shoot Length (mm)	31.4	49.0	45.8	58.8	3.7	11.2		
Inhibition (%)		-56.1	-79.9	-87.3				

Values in parentheses are angular values

The results of bioassay studies of <u>C</u>. <u>fulvus</u> spikelet extract on <u>V</u>. <u>radiatus</u> seeds also showed that there was no inhibitory effect of leachate on germination percent of <u>V</u>. <u>radiatus</u>. The maximum germination of 88.8% was recorded with 50% conc. (Table 15 b Fig. 5B, Plate 10). However, significant inhibition on percentage root length was recorded (Appendix V b) as the concentration of leachate increased from 25 to 100% concentration. The maximum reduction (51.6%) in root length was recorded at 100% concentration. Data on shoot length revealed that the 7.6% inhibition was recorded with 25% concentration and 9.7% was observed with 100% concentration. But at 50% concentration inhibitory effect was not observed.

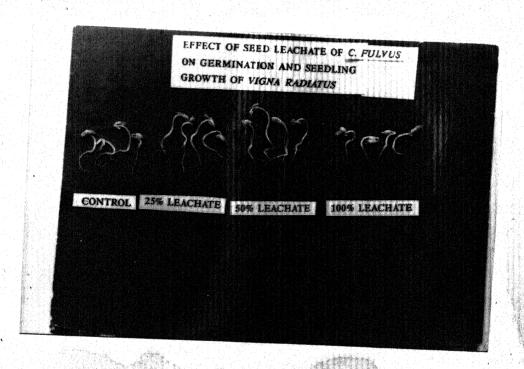


PLATE 10: EFFECT OF *C. FULVUS* DIASPORE EXTRACT ON GERMINATION AND GROWTH OF ROOT AND SHOOT OF *V. RADIATUS* 



Table 15b: Effect of <u>C. fulvus</u> Diaspore Extract on Germination and Growth of Root and Shoot of <u>Vigna radiatus</u>

) (14 ) (14) (14) (14) (14) (14) (14) (1	CONCENTRATION OF LEACHATE OF <u>C</u> . <u>fulvus</u>						
	Control	25% Conc.	50% Conc.	100% Conc.	SEm <u>+</u>	CD 5%	
Germination (%)	80.8 (64.0)	86.4 (68.5)	88.8 (70.6)	81.6 (64.7)	(1.09)	(3.4)	
Inhibition (%)	-	-6.9	-9.9	-0.9			
Root Length (mm)	36.0	28.8	19.4	17.4	0.8	2.4	
Inhibition (%)	-	20.0	46.1	51.6			
Shoot Length (mm)	28.8	26.6	28.8	26.0	2.1	6.5	
Inhibition (%)	_	7.6	0	9.7			

The results of bioassay studies of <u>C</u>. <u>fulvus</u> spikelet extract on <u>C</u>. <u>fulvus</u> seed (caryopsis) showed significant variation in germination with different concentration of leachate (Appendix V C). However, at 25% ccnc. there was no inhibitory effect on germination but as the concentration increases the percentage germination showed higher inhibitory effect (Table 15 c, Fig.5C). The maximum (61.7%) percentage of inhibitory effect was observed at 100% concentration. The root length of <u>C</u>. <u>fulvus</u> significantly reduced as the concentration enhanced from 25 to 100%. The hundred percent inhibition in root length was recorded at 100% conc. In case of shoot length it was observed that at 25 and 50% concentrations, there was stimulatory effect but at 100% concentration inhibitory effect recorded was 62.1%.

Table 15c: Effect of <u>C</u>. <u>fulvus</u> Diaspore Extract on Germination and Growth of Root and Shoot of <u>C</u>. <u>fulvus</u>

	CON	CONCENTRATION OF LEACHATE OF C. fulvus						
	Control	25% Conc.	50% Conc.	100% Conc.	SEm <u>+</u>	CD 5%		
Germination (%)	24.0 (29.3)	34.4 (35.9)	21.6 (27.6)	9.2 (19.4)	(2.6)	(8.1)		
Inhibition (%)	_	-43.3	10.0	61.7				
Root Length (mm)	23.0	17.0	4.8	0	1.6	4.8		
Inhibition (%)	-	26.1	79.1	100.0				
Shoot Length (mm)	44.8	54.0	47.2	17.0	2.9	9.1		
Inhibition (%)		-20.5	-5.4	62.1	·			

#### Pennisetum pedicellatum:

The bioassay study with the water extract of <u>P</u>. <u>pedicellatum</u> spikelets on percent germination as well as root and shoot growth of <u>R</u>. <u>sativus</u> and percentage inhibition of germination and root and shoot growth have been presented in table 16 a (Fig. 6A). It was observed that percentage of inhibition of <u>R</u>. <u>sativus</u> increased with increasing concentration of leachate of <u>P</u>. <u>pedicellatum</u>. The maximum inhibition of 10.9% was recorded with 100% concentration. Data on length of root showed that there was significant decrease in root length as the concentration of leachate (Appendix VI a) enhanced from 25 to 100% concentration, but there was no inhibitory effect of leachate on shoot length of <u>R</u>. <u>sativus</u> even up to 100% concentration. This showed that there was no stimulatory effect on shoot length of R. sativus.

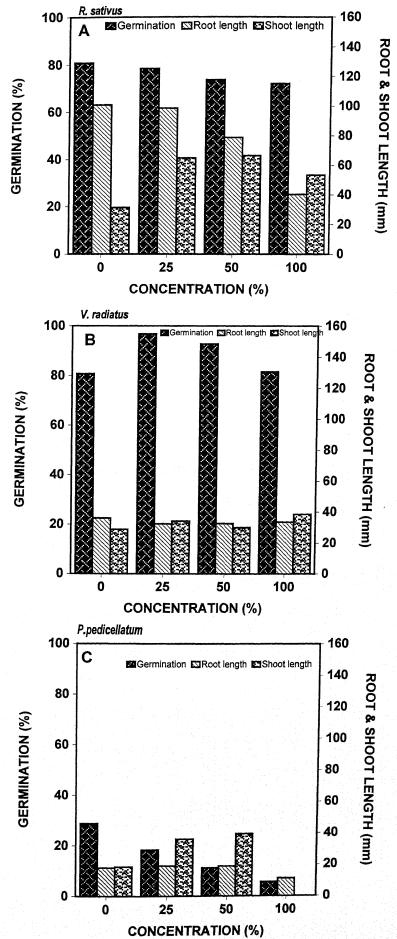


Fig. 6 (A-C). Effect of *P. pedicellatum* diaspore extract on germination and growth of root and shoot of *R. sativus* (A), *V. radiatus* (B) and *P. pedicellatum* (C).

Table 16a: Effect of <u>P. pedicellatum</u> Diaspore Extract on Germination and Growth of Root and Shoot of <u>R. sativus</u>

	CONCE	CONCENTRATION OF LEACHATE OF P. pedicellatum						
o	Control	25% Conc.	50% Conc.	100% Conc.	SEm <u>+</u>	CD 5%		
Germination (%)	80.8 (64.0)	78.4 (62.3)	73.6 (59.1)	72.0 (61.7)	(1.6)	(4.8)		
Inhibition (%)		2.97	8.9	10.9				
Root Length (mm)	100.8	98.6	78.8	40.4	20.1	61.8		
Inhibition (%)		2.1	21.8	59.9				
Shoot Length (mm)	31.4	65.0	66.6	53.4	3.7	11.4		
Inhibition (%)	-	-107.0	-112.1	-70.1				

The results of bioassay studies of <u>P</u>. <u>pedicellatum</u> spikelets extract on <u>V</u>. <u>radiatus</u> seeds also showed that there was no inhibitory effect of leachate on germination percent of <u>V</u>. <u>radiatus</u>. The maximum germination of 96.8% was recorded with 25% concentration (Table 16 b,Fig. 6B, Plate 11). Significant inhibition on percent root length was recorded (Appendix. VI b). However, maximum inhibition of 10.0% was noted with 25% concentration followed by 50 and 100% concentration. Data on shoot length showed that there was no inhibitory effect of leachate on shoot length of <u>V</u>. <u>radiatus</u>. The maximum shoot length of 38.6 mm was observed with 100% concentration.

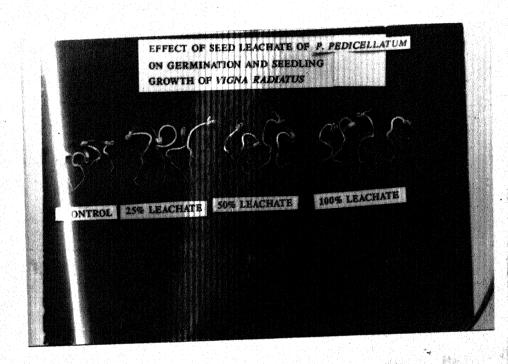


PLATE 11: EFFECT OF *P. PEDICELLATUM* DIASPORE EXTRACT ON GERMINATION AND GROWTH OF ROOT AND SHOOT OF *V. RADIATUS* 

Table 16b: Effect of P. pedicellatum Diaspore Extract on Germination and Growth of Root and Shoot of V. radiatus

	CONCE	CONCENTRATION OF LEACHATE OF P. pedicellatum						
	Control	25% Conc.	50% Conc.	100% Conc.	SEm±	CD 5%		
Germination (%)	80.8 (64.1)	96.8 (84.1)	92.8 (74.8)	81.6 (64.9)	(2.3)	(7.1)		
Inhibition (%)	_	-19.8	-14.9	-0.9				
Root Length (mm)	36.0	32.4	32.6	33.6	1.3	3.9		
Inhibition (%)	_	10.0	9.4	6.7				
Shoot Length (mm)	28.8	34.2	30.0	38.6	3.4	10.4		
Inhibition (%)	_	-18.8	-4.2	-34.0				

The result of bioassay studies of <u>P</u>. <u>pedicellatum</u> spikelets extract on <u>P</u>. <u>pedicellatum</u> seeds (caryopsis) showed significant variation in germination with different concentration of leachate (Appendix VI C). The concentration increase of leachate showed higher inhibitory effect (Table 16 c, Fig. 6C). The maximum (80.5%) percentage of inhibitory effect was observed at 100% concentration. Data on length of root showed that there was no inhibitory effect of <u>P</u>. <u>pedicellatum</u> leachate even upto 50% concentration. However, 37.8% inhibition was recorded at 100% concentration. In case of shoot length there was no inhibitory effect of leachate of shoot length of <u>P</u>. <u>pedicellatum</u> leachate even upto 50% concentration, but 100% inhibition was noted at 100% concentration. The maximum shoot length of 39.6 mm was observed with 50% concentration.

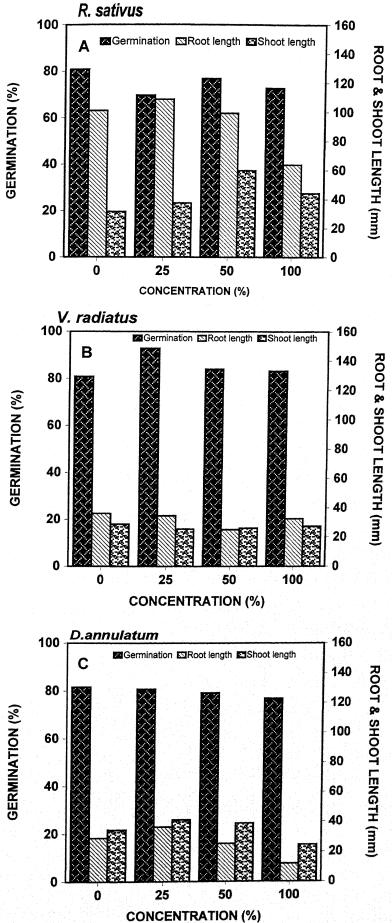
Table 16c: Effect of P. pedicellatum Diaspore Extract on Germination and Growth of Root and Shoot of P. pedicellatum

	CONCE	CONCENTRATION OF LEACHATE OF P. pedicellatum						
	Control	25% Conc.	50% Conc.	100% Conc.	SEm <u>+</u>	CD 5%		
Germination (%)	28.8 (32.4)	18.4 (25.3)	11.2 (19.4)	5.6 (14.5)	(4.5)	(13.7)		
Inhibition (%)	_	36.1	61.1	80.5				
Root Length (mm)	18.0	19.2	19.0	11.2	2.8	8.6		
Inhibition (%)	_	-6.7	-5.6	37.8				
Shoot Length (mm)	18.6	36.2	39.6	0	11.3	34.9		
Inhibition (%)	_	-94.6	-112.9	-100.0				

#### Dichanthium annulatum:

The result of bioassay studies with the water extract of spikelets of <u>D</u>. <u>annulatum</u> on the germination as well as root and shoot growth of <u>R</u>. <u>sativus</u> has been given in table 17 a (Fig. 7A). It was observed that there was inhibitory effect of leachate on the germination of <u>R</u>. <u>sativus</u> and it was maximum i.e. 13.9% at 25% concentration.

Data on root length showed that there was no inhibitory effect of root length at 25% concentration but inhibitory effect was observed at 50 and 100% concentration. As regards to shoot length there was significant differences due to different concentration of leachate (Appendix. VII a) and maximum length of 59.8 mm was recorded with 50% concentration. However, there was no inhibitory effect of leachate in shoot length at any concentration which showed that there was stimulatory effect.



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3./OAN Fig 7 (A-C). Effect of *D. annulatum* diaspore exract on germination and growth of root and shoot of R. sativus (A), V. radiatus (B) and D. annulatum (C).

Table 17a: Effect of <u>D</u>. <u>annulatum</u> Diaspore Extract on Germination and Growth of Root and Shoot of <u>R</u>. <u>sativus</u>.

	CONC	CONCENTRATION OF LEACHATE OF D. annulatum						
•	Control	25% Conc.	50% Conc.	100% Conc.	SEm <u>+</u>	CD 5%		
Germination (%)	80.8 (64.0)	69.6 (56.9)	76.8 (61.3)	72.8 (58.6)	(1.4)	(4.2)		
Inhibition (%)	-	13.9	4.9	9.9				
Root Length (mm)	100.8	108.6	99.2	63.8	3.9	12.0		
Inhibition (%)	_	-7.7	1.6	36.7				
Shoot Length (mm)	31.4	37.4	59.8	44.2	5.5	16.8		
Inhibition (%)	•	-19.1	-90.4	-40.8				

The results of bioassay study of <u>D</u>. <u>annulatum</u> spikelets extract on <u>V</u>. <u>radiatus</u> seeds showed that there was no inhibitory effect of leachate on germination percent of <u>V</u>. <u>radiatus</u>. The maximum germination of 92.8% was recorded with 25% concentration (Table 17 b, Fig. 7B, Plate 12). Significant inhibition on percent root

Table 17b: Effect of <u>D</u>. <u>annulatum</u> Diaspore Extract on Germination and Growth of Root and Shoot of <u>V</u>. <u>radiatus</u>

	CONC	ENTRATI	ON OF LE	ACHATE	OF <u>D. anı</u>	D. <u>annulatum</u>			
0	Control	25% Conc.	50% Conc.	100% Conc.	SEm±	CD 5%			
Germination (%)	80.8 (64.0)	92.8 (74.7)	84.0 (66.5)	83.2 (65.8)	(1.4)	(4.4)			
Inhibition (%)	4	-14.9	3.9	-2.9					
Root Length (mm)	36.0	34.6	25.0	32.6	1.1	3.2			
Inhibition (%)		3.9	30.6	9.4					
Shoot Length (mm)	28.8	25.4	26.0	27.4	3.4	10.4			
Inhibition (%)		11.8	9.7	4.9					

Values in parentheses are angular values

length was recorded (Appendix VII b). However, maximum inhibition of 30.6% was noted

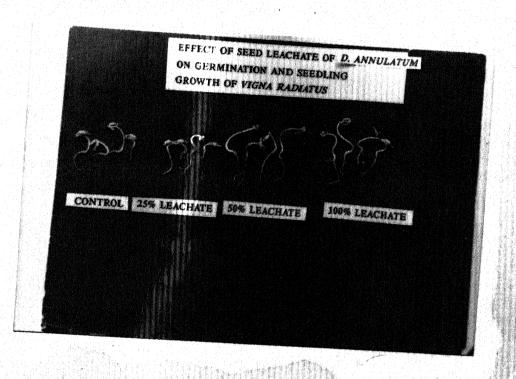


PLATE 12: EFFECT OF D. ANNULATUM DIASPORE
EXTRACT ON GERMINATION AND GROWTH
OF ROOT AND SHOOT OF V. RADIATUS

with 50% concentration followed by 100 and 25% concentration. Data on shoot length showed that significant inhibition of leachate was recorded. The maximum inhibition of 11.8% was noted with 25% concentration.

The results of bioassay studies of <u>D</u>. <u>annulatum</u> spikelets extract on <u>D</u>. <u>annulatum</u> seeds (caryopsis) showed significant variation in germination with different concentrations of leachate (Appendix VII c). The concentration causing increase in the percentage germination showed higher inhibitory effect (Table 17c, Fig. 7C). The

Table 17c: Effect of <u>D</u>. <u>annulatum</u> Diaspore Extract on Germination and Growth of Root and Shoot of <u>D</u>, <u>annulatum</u>

	CONC	CONCENTRATION OF LEACHATE OF D. annulatum							
	Control	25% Conc.	50% Conc.	100% Conc.	SEm <u>+</u>	CD 5%			
Germination (%)	81.6 (64.6)	80.8 (64.9)	79.2 (65.3)	76.8 (61.3)	(2.0)	(6.2)			
Inhibition (%)	_	0.9	2.9	5.9					
Root Length (mm)	29.2	36.6	25.4	12.0	2.0	6.3			
Inhibition (%)	-	-25.3	13.0	58.9					
Shoot Length (mm)	34.6	41.4	39.0	24.6	1.9	6.1			
Inhibition (%)	-	-19.7	-12.7	28.9					

Values in parentheses are angular values

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maximum (5.9%) percentage of inhibition was observed at 100% concentration. The root length of <u>D</u>. annulatum as well as percent germination showed no inhibitory effect at 25% concentration, but as the concentration increases the percentage germination showed higher inhibitory effect. The maximum of 58.9% inhibitory effect was observed at 100% concentration. In case of shoot length it was observed that at 25 and 50% concentration there was stimulatory effect but at 100% concentration inhibitory effect was recorded (28.9%).

#### Bothriochloa intermedia:

The results of bioassay study with the water extract of spikelets of  $\underline{B}$ . intermedia on the germination as well as root and shoot growth of  $\underline{R}$ . sativus have been given in table 18a (Fig. 8A). It was observed that there was no inhibitory effect of  $\underline{B}$ . intermedia

Table 18a: Effect of <u>B</u>. <u>intermedia</u> Diaspore Extract on Germination and Growth of Root and Shoot of <u>R</u>. <u>sativus</u>

	CONC	CONCENTRATION OF LEACHATE OF B. intermedia						
	Control	25% Conc.	50% Conc.	100% Conc.	SEm <u>+</u>	CD 5%		
Germination (%)	88.8 (64.0)	92.8 (74.7)	89.6 (71.6)	76.0 (60.7)	(1.4)	(4.4)		
Inhibition (%)	_	-14.9	-10.9	5.9				
Root Length (mm)	100.8	132.2	144.0	94.2	5.0	15.6		
Inhibition (%)		-31.2	-42.9	6.5				
Shoot Length (mm)	31.4	56.8	54.6	53.0	2.5	7.8		
Inhibition (%)		-80.9	-73.9	-68.8				

Values in parentheses are angular values

leachate even up to 50% concentration. However, 5.9% inhibition was recorded at 100% concentration. Data on root and shoot length showed significant variation (Appendix VIII a). In case of root length it was observed that at 25 and 50% concentration, there was stimulatory effect but at 100% concentration, inhibitory effect was recorded (6.5%). As regards of shoot length there was no inhibitory effect of leachate of  $\underline{R}$ . sativus even upto 100% concentration. This showed that there was stimulatory effect on shoot length of  $\underline{R}$ . sativus.

The results of bioassay study of <u>B</u>. <u>intermedia</u> spikelets extract on <u>V</u>. <u>radiatus</u> seeds showed significant variation in germination (Appendix VIII b, Table 18 b, Fig. 8B, Plate 13). It was observed that there was no inhibitory effect of <u>B</u>. <u>intermedia</u> leachate even up to 50% concentration. However, 9.9% inhibition was recorded at 100% concentration. Data on root

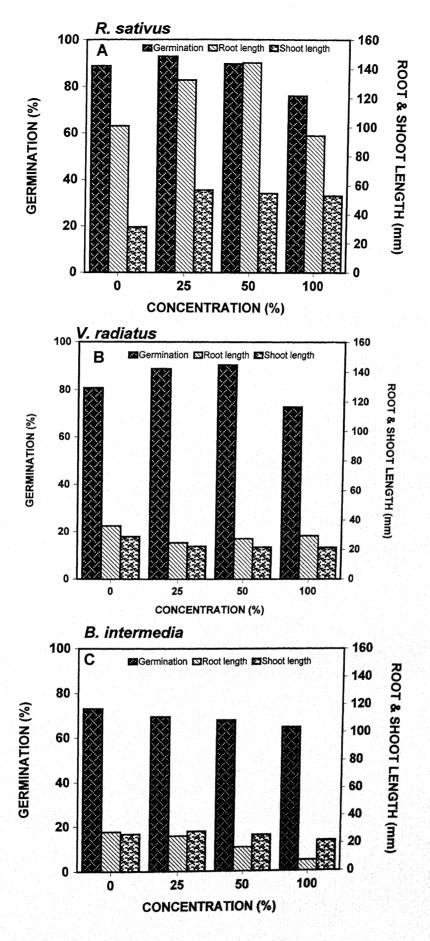


Fig 8 (A-C). Effect of *B. intermedia* diaspore extract on germination and growth of root and shoot of *R. sativus* (A), *V. radiatus* (B) and *B. intermedia* (C).

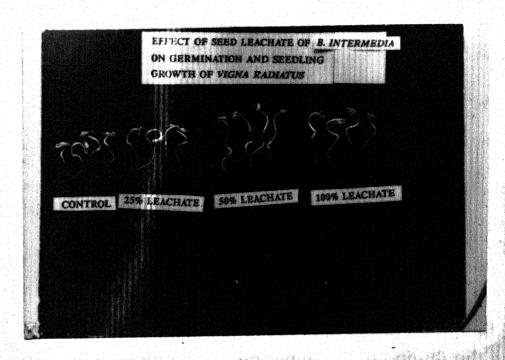


PLATE 13: EFFECT OF *B. INTERMEDIA* DIASPORE
EXTRACT ON GERMINATION AND GROWTH
OF ROOT AND SHOOT OF *V. RADIATUS* 

and shoot length also showed significant variation. In case of root length there was significant increase as the concentration of leachate enhanced from 25 to 100%. The minimum inhibition of 18.3% was noted at 100% concentration. As regards of shoot length the minimum inhibition (22.9%) was recorded at 25% concentration.

Table 18b: Effect of <u>B</u>. <u>intermedia</u> Diaspore Extract on Germination and Growth of Root and Shoot of <u>V</u>. <u>radiatus</u>.

	CONC	CONCENTRATION OF LEACHATE OF B. intermedia							
	Control	25% Conc.	50% Conc.	100% Conc.	SEm±	CD 5%			
Germination (%)	80.8 (64.0)	88.8 (68.8)	90.4 (71.6)	72.8 (78.7)	(8.4)	(25.9)			
Inhibition (%)	-	-9.9	-11.9	9.9					
Root Length (mm)	36.0	24.6	27.4	29.4	1.1	3.4			
Inhibition (%)	-	31.7	23.8	18.3	:				
Shoot Length (mm)	28.8	22.2	21.6	21.4	2.3	7.2			
Inhibition (%)	•	22.9	25.0	25.7					

Values in parentheses are angular values

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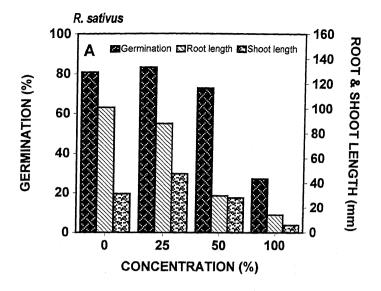
The results of bioassay study of <u>B</u>. <u>intermedia</u> spikelets extract on <u>B</u>. <u>intermedia</u> seeds (caryopsis) showed significant variation in germination with the different concentration of leachate (Appendix VIII c). The leachate concentration causing increase in percent germination showed higher inhibitory effect (Table 18 c, Fig. 8C). The maximum percentage (11.5%) of inhibitory effect was observed at 100% concentration. Data on length of root showed that there was significant decrease in root length as the concentration of leachate enhanced from 25 to 100% concentration. In case of shoot length, there was no inhibitory effect of shoot length at 25% concentration but significant inhibition was recorded at 100% concentration. The maximum inhibition of 18.7% was noted with 100% concentration.

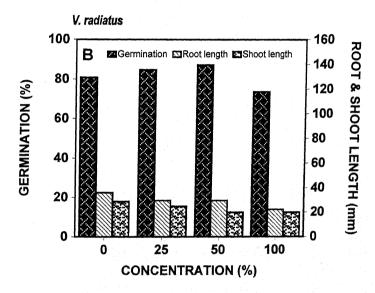
Table 18c: Effect of <u>B</u>. <u>intermedia</u> Diaspore Extract on Germination and Growth of Root and Shoot of <u>B</u>. <u>intermedia</u>

	Concentration of Leachate of <u>B</u> . <u>intermedia</u>					
	Control	25% Conc.	50% Conc.	100% Conc.	SEm <u>+</u>	CD 5%
Germination (%)	73.2 (58.9)	69.6 (56.5)	68.0 (55.7)	64.8 (53.6)	(1.8)	(5.5)
Inhibition(%)	_	4.9	7.1	11.5		
Root length (mm)	28.4	25.4	17.2	7.8	1.0	3.1
Inhibition (%)	_	10.6	39.4	72.5		
Shoot length (mm)	26.8	28.6	26.0	21.8	2.1	6.4
Inhibition (%)	_	-6.7	2.9	18.7		

#### Panicum maximum:

The results of bioassay study with the water extract of spikelets of <u>P. maximum</u> on the germination as well as root and shoot growth of <u>R. sativus</u> has been given in Table 19 a, Fig. 9A). It showed significant variation in germination with different concentration of leachate (Appendix IX a). However at 25% concentration there was no inhibitory effect on germination but as the concentration increases the percentage germination showed higher inhibitory effect. The maximum (66.3%) percentage of inhibitory effect was observed at 100%, concentration. Data on root length of <u>P. maximum</u> reduced significantly. As the concentration increases from 25 to 100% inhibition increases too. The maximum inhibition of 85.9% was recorded at 100%





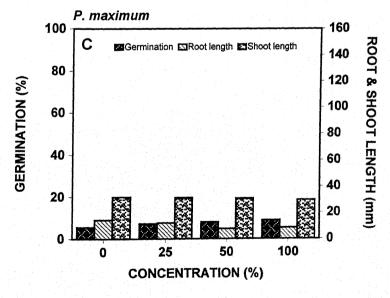


Fig 9 (A-C). Effect of *P. maximum* diaspore extract on germination and growth of root and shoot of *R. sativus* (A), *V. radiatus* (B) and *P. maximum* (C).

Table 19a: Effect of <u>P</u>. <u>maximum</u> Diaspore Extract on Germination and Growth of Root and Shoot of <u>R</u>. <u>sativus</u>.

	CONCENTRATION OF LEACHATE OF P. maximum					
	Control	25% Conc.	50% Conc.	100% Conc.	SEm±	CD 5%
Germination (%)	80.8 (64.0)	83.2 (65.8)	72.8 (58.5)	27.2 (30.8)	(2.3)	(6.9)
Inhibition (%)		-2.9	9.9	66.3		
Root Length (mm)	100.8	87.8	29.8	14.2	4.1	12.7
Inhibition (%)	-	12.9	70.4	85.9		
Shoot Length (mm)	31.4	47.6	28.2	6.0	3.9	12.1
Inhibition (%)	-	-51.6	10.2	80.9		

concentration. In case of shoot length of <u>P</u>. <u>maximum</u> there was no inhibitory effect at 25% concentration but 10.2 and 80.9% inhibition was recorded at 50 and 100% concentrations, respectively.

The results of bioassay study of P. maximum spikelets extract on V. radiatus seeds showed that there was no inhibitory effect of P. maximum leachate even upto 50% concentration. However, 8.9% inhibition was recorded at 100% concentration (Table 19 b,Fig. 9B, Plate 14). Data on length of root showed significant inhibition and maximum inhibition i.e. 38.3% was observed at 100% concentration, Similarly significant inhibitory effect was observed on shoot length (Appendix IX b). However, inhibitory effect on 50 to 100% concentration was at par.

The results of bioassay study of <u>P</u>. <u>maximum</u> spikelets extract on <u>P</u>. <u>maximum</u> seeds (caryopsis) showed no inhibitory effect of leachate on germination percent of <u>P</u>. <u>maximum</u>. The maximum germination of 8.8% was recorded with 100% concentration (Table 19 c, Fig. 9C).

Table 19b: Effect of P. maximum Diaspore Extract on Germination and Growth of Root and Shoot of V. radiatus

	CONCENTRATION OF LEACHATE OF P. maximum					
ī	Control	25% Conc.	50% Conc.	100% Conc.	SEm±	CD 5%
Germination (%)	80.8 (64.0)	84.8 (67.1)	87.2 (69.2)	73.6 (59.2)	(1.4)	(4.3)
Inhibition (%)	-	-4.9	-7.9	8.9		
Root Length (mm)	36.0	29.6	29.6	22.2	1.2	3.6
Inhibition (%)	. <b>-</b>	17.8	17.8	38.3		
Shoot Length (mm)	28.8	24.8	19.8	19.8	2.6	7.9
Inhibition (%)	_	13.8	31.3	31.3		

Table 19c: Effect of <u>P. maximum</u> Diaspore Extract on Germination and Growth of Root and Shoot of <u>P. maximum</u>

	CONCENTRATION OF LEACHATE OF P. maximu					
	Control	25% Conc.	50% Conc.	100% Conc.	SEm <u>+</u>	CD 5%
Germination (%)	5.6 (13.5)	7.2 (15.2)	8.0 (16.2)	8.8 (16.9)	(3.9)	(12.3)
Inhibition (%)		-28.6	-42.9	-57.1		
Root Length (mm)	14.4	12.2	7.6	8.4	1.0	3.1
Inhibition (%)		15.3	47.2	41.7		
Shoot Length (mm)	32.0	31.4	30.8	29.6	3.6	11.1
Inhibition (%)		1.9	3.8	7.5		

Values in parentheses are angular values

Significant inhibition on root length was recorded (Appendix IX C). However, maximum inhibition of 47.2% was noted with 50% concentration followed by 100 and 25% concentration. Data on shoot length showed significant inhibition and the maximum inhibition of 7.5% was noted with 100% concentration.

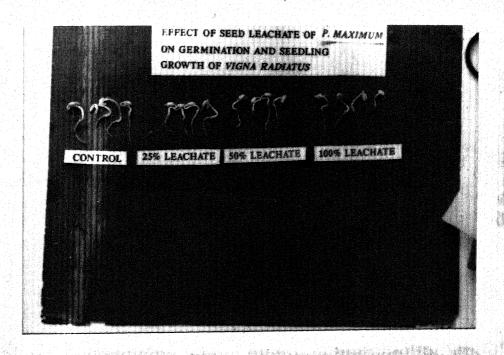


PLATE 14: EFFECT OF *P. MAXIMUM DIASPORE*EXTRACT ON GERMINATION AND GROWTH
OF ROOT AND SHOOT OF *V. RADIATUS* 

## **DISCUSSION**

Studies on various aspects of seed germination viz., dormancy, scarification, storage, rate, isolation and characterisation of inhibitors and bioassay were conducted and the results obtained are discussed in the ensuing paragraphs.

## 1. Dormancy mechanisms:

A freshly collected spikelets as well as seeds of 3 grasses viz., <u>P. pedicellatum</u>, <u>B. intermedia</u> and <u>P. maximum</u> showed dormancy except in seeds of <u>B. intermedia</u> which showed 12.4% germination (Table 4). Further, it was observed that incase of <u>P. maximum</u> dormancy remain ad upto three months both in spikelets and seeds (Table 6a, b).

Such type of dormancy has also been observed by several workers in different grasses and cereals (Toole, 1939, 1940 a,b; Toole and Toole, 1941; Schwendiman and Shands, 1943; Crosier, 1946; Brown et. al. 1948; Lang, 1965; Mott, 1974; Renard and Capellel, 1976; Pathak et. al. 1976; Tothill, 1977; Junttila, 1977; Whiteman and Mendra, 1982; Ackigoz and Knowles, 1983; Parihar, et. al. 1984 a,b; Matias and Bilbao, 1985; Selvaraj and Ramaswamy, 1986;. Morgan and Myers, 1989; West and Marousky, 1989; Nancy and Boe, 1991; Hardegree and Emmerich, 1991 and many others.

This inability of freshly harvested seeds to germinate has been termed as primary dormancy. The seeds come out of primary dormancy during the process after ripenning (Bewley and Black, 1985). However, in case of <u>C</u>. <u>fulvus</u> and <u>D</u>. <u>annulatum</u> freshly harvested seeds were able to germinate indicating that there is no primary dormancy. Many grass species viz., <u>Lolium</u>, <u>Bromus</u> etc. common in European meadows and pastures are also reported of being capable of immediate germination (Bewley and Black,

1985). Freshly harvested spikelets of <u>Cenchrus ciliaris</u> are also capable of immediate germination (Pandeya and Jayan, 1978; Parihar, <u>et. al.</u> 1984 b). The results obtained in this studies in case of <u>C.fulvus</u> and <u>P. pedicellatum</u> are contradictory to the results reported by Parihar, 1988.

Removal of seeds from the enclosing glumes have an enhanced effect on percentage germination as well as rate of germination (Table 7, Fig.4). Such type of observations have also been reported by Parihar, et. al. 1984 in C. setigerus, Weaver and Jordan, 1985 and Parihar and Kanodia, 1993 in Bothriochloa species. However, the effectiveness of this treatment was considerably reduced with increasing the storage periods, indicating that there is a secondary dormancy in operation, due to endogenous as well as exogenous inhibitors. The endogenous inhibitors are present in the naked caryopsis (seed) which are labile with the time decreasing in their effect considerably during the storage. The exogenous inhibitors are associated with seeds enclosing glumes, lemmas, paleas etc. which are also labile with the time. A third factor that also probably controls the germ nation of spikelets are some kinds of mechanical restrain imposed by glumes over the seed. The mechanical restriction also possibly prevent the leaching of the inhibitors from the caryopsis and also reduced the oxygen transport to embryo. Mott, 1974; Tothill, 1977; Thornas and Allison, 1975; Juntilla, 1977; Whiteman and Mendra, 1982 have outlined a similar dual dormancy system in a number of grass species. Mott (1974) also concluded that dormancy associated with the seed hull of Aristida contorta is apparently due to mechanical restriction of gas (oxygen) exchange by hull. Anatomical studies by Mott and Tynan (1974) identified lipid containing layer on the inner surface of the hull of Aristida contorta which was intact in dormant grains but fractured in non-dormant grains.

Martin (1975) suggested that the dormancy in <u>Themeda triandra</u> is associated with a general increase in the growth potential of the embryo, which overcomes the mechanical resistance of the glumes to germination over a period of 12 months. Hagon (1976) supported the idea that the glumes, lemmas etc. were a mechanical barrier to germination, whose effect is modified by changing physiology in the embryo. Hagon (1976) also believed that seed enclosing structures also control dormancy in <u>Themeda</u> by containing most of the inhibitory substances, while in <u>Stipa</u> the role of lemmas and palea was two folds i.e. firstly, they contain inhibitors whose effect could be overcome by gibberellic acid and secondly, the role of lemmas and palea was to either mechanically restrict germination, reduce oxygen transport to embryo, prevent the leaching of the inhibitors etc.

Thus, in the whole dispersal unit, all the three controlling systems seem to operate simultaneously. As a result, the percentage germination as well as the rate of germination is low.

### Significance of dormancy:

Under natural conditions the initiation of growth in most of these range grasses takes place at the onset of mansoon and seeds (diaspores) are shed from September to December. The presence of inhibitors limit the germination immediately after seed fall even though suitable temperature and moisture conditions may be available. These mechanisms seem to prevent the loss of seedlings owing to following low temperature as well as low moisture conditions in winters. Several months after the seed fall the level of inhibitors would be reduced due to oxidation owing to high summer temperature or due to labile nature of inhibitors and as a result of after ripening, the new seedlings will emerge only at the advent of monsoon. Such seedlings would be expected to establish

satisfactorily. Mott (1972) found that seed dormancy and temperature were major factors which determined the seasonal flora in arid regions of Western Australia.

## 2. Effect of Scarification Treatments:

<u>Pre-chilling</u>: The results indicated that the pre-chilling treatment has increased the germination percentage in freshly collected seeds incase of <u>C</u>. <u>fulvus</u> and <u>B</u>. <u>intermedia</u> while there is no effect of pre-chilling in case of <u>P</u>. <u>pedicellatum</u>, <u>D</u>. <u>annulatum</u> and <u>P</u>. <u>maximum</u> in germination (Table 5 a, Fig. 2A).

In case of 9 months stored seeds, enhanced germination was observed in <u>D</u>. annulatum only while in other four grasses there was no beneficial effect of pre-chilling treatment on germination. The beneficial effect of pre-chilling treatment for enhancing germination in fresh and stored seeds has also been reported by many workers in some of the grasses (Tool, 1939, 1940, a,b, 1941; Kearns and Toole, 1939; Toole and Toole, 1941; Stokes, 1965; Junttila, 1977; Whiteman and Mendra, 1982; Bewley and Black, 1985 and Parihar, 1988.

The reasons of dormancy reduction by chilling is not fully understood. However, according to Bewley and Black (1985) low temperature lowers the enzymatic reaction taking place in the seed which adversely affect the dormancy imposing mechanisms. According to Simpson (1965); Wareing et al. (1972) and Mayer and Poljakoff-Maber (1982) chilling changes the balance of growth substances in a seed considerably.

## Hot Water and heat Treatments:

The results indicated that the hot water treatment slightly increased the germination percentage in freshly collected seeds in case of <u>C</u>. <u>fulvus</u> only while there was no effect of

hot water in case of <u>P</u>. <u>pedicellatum</u>, <u>D</u>. <u>annulatum</u>, <u>B</u>. <u>intermedia</u> and <u>P</u>. <u>maximum</u> (Table 5a, Fig.2A).

In case of 9 months stored seeds marginal enhanced germination was observed in P. annulatum while in other three grasses (C. fulvus, B. intermedia and P. maximum) there was no beneficial effect of hot water treatment on germination (Table 5b, Fig.2B).

As regards to heat treatment enhanced germination was observed in freshly collected seeds in case of <u>C</u>. <u>fulvus</u> and <u>B</u>. <u>intermedia</u> while there was no effect of heat treatment in case of <u>P</u>. <u>pedicellatum</u>, <u>D</u>. <u>annulatum</u> and <u>P</u>. <u>maximum</u> (Table 5a, Fig.2A).

Heat treatment at 9 months stored seed showed higher germination in P. pedicellatum and D. annulatum while in other 3 grasses no beneficial effect of seeds (Table 5b, Fig. 25) through brief exposure to slightly elevated temperatures which is common in many kinds of seeds including grasses (Stokes, 1965; Mayer and Poljakoff-Mayber, 1982). Sampson (1944) showed that the seeds of grasses could tolerate temperature of 82° to 116° C for five minutes and these temperature exposures usually increase the percentage of germination. In some cases, seeds harvested from recently burned grassland were found to have higher percentage of germination than those of nearly unburnt grassland (Ehrenrei ch and Aikman, 1963; grant et. al., 1963).

The mechanism of the effect of raised temperature is in no way clear (Mayer and Roljakoff-Mayber, 1982). However, Hendricks and Taylorson (1979) brought direct evidence for changes in properties of seed membranes of <u>Barbarea verna</u> and lettuce at well defined, critical temperatures. Membrane fractions were prepared, labeled with a temperature sensitive probe and the effect of temperature on such membrane was then determined. The critical temperature at which the fluidity of the membrane occurred

coincided with the temperature at which physiological response of the seeds also occurred.

Parihar (1988) reported that dormancy of freshly collected spikelets could be reduced with hot water treatment incase of <u>C</u>. <u>fulvus</u> and <u>B</u>. <u>intermedia</u>. Similarly heat treatment also reduced dormancy incase of <u>C</u>. <u>fulvus</u> and <u>P</u>. <u>pedicellatum</u>. However, he observed that incase of nine months stored spikelets there was no beneficial effect of hot water and heat treatment in case of <u>C</u>. <u>fulvus</u>, <u>B</u>. <u>intermedia</u> and <u>P</u>. <u>pedicellatum</u>.

#### Effect of Ethanol:

None of the five grass species studied showed beneficial effect of ethanol solution to break dormancy since there was no germination. However, some of the authors reported beneficial effect of ethanol solution on freshly collected seeds of grasses for breaking the dormancy (Mayer and Poljakoff -Mayer, 1982; Bewley and Black, 1985 and Parihar, 1988).

#### Effect of Potassium nitrate:

The results indicated that the potassium nitrate treatment marginally increased the germination percentage in freshly collected seeds in case of <u>C</u>. <u>fulvus</u> and <u>D</u>. <u>annulatum</u> while there was no effect on <u>P</u>. <u>pedicellatum</u>. <u>B</u>. <u>intermedia</u> and <u>P</u>. <u>maximum</u> (Table 5a, Fig. 2B).

In case of nine months stored seed, enhanced germination was observed in <u>C</u>. fulvus, <u>P</u>. pedicellatum and <u>D</u>. annulatum while in the two grasses (<u>B</u>. intermedia and <u>P</u>. maximum) there was no beneficial effect of potassium nitrate treatment on germination (Table 5b, Fig. 2B). Potassium nitrate was one of the first chemical material which was

found to overcome certain causes of dormancy in cereals and grasses (Stokes, 1965). Many workers have also reported that potassium nitrate was effective in reducing the dormancy in cereals and grasses (Schwendiman and Shands, 1943; Anderson, 1944; Kearns and Toole, 1939; Toole, 1939, 1941; Hagon, 1976; Tothill, 1977; Whiteman and Mendra, 1982; Parihar, 1988; Basra, et. al. 1990; Toledo and Carbalho, 1990; Yoledo, et. al. 1994; and Khandelwal and Sen, 1994).

## Effect of Gibberellic acid:

The results indicated that the gibberellic acid treatment gave significantly higher germination percentage in freshly collected seeds in case of <u>C</u>. <u>fulvus</u> and <u>D</u>. <u>annulatum</u> while there was no effect on the seeds of <u>P</u>. <u>pedicellatum</u>, <u>B</u>. <u>intermedia</u> and <u>P</u>. <u>maximum</u> (Table 5a ,Fig. 2A).

In case of 9 months stored seeds significant increase in germination was observed in <u>D</u>. annulatum vihile in other four grasses (<u>C</u>. fulvus, <u>P</u>. pedicellatum, <u>B</u>. intermedia and <u>P</u>. maximum) there was no beneficial effect of gibberellic acid treatment on germination (Table 5b, Fig. 2E). Enhanced germination in cereals and grasses due to gibberellic acid treatment is well known from several earlier experiments. For example, Nakamura <u>et</u>. <u>al</u>. (1960) observed enhanced germination due to gibberellic acid in <u>Poa</u>, <u>Cynodon</u> and <u>Festuca</u> species. Hagon (1976) reported reduction in dormancy by gibberellic acid in <u>Themeda australis</u>, <u>Danthonia</u>, <u>Corphoides</u>, <u>Stipa bigeneculata</u> and <u>Bothriochloa macra</u>. Tothill (1977) four d reduction of dormancy in <u>Heteropogon contortus</u>. Junttila (1977) and Whiteman and Mendra (1982) observed reduction in dormancy as well as enhanced germination in stored seeds of <u>Dactylis glomerata</u> and <u>Brachiaria decumbens</u>.

In case of cereals also, gibberellic acid has been found to be effective in reducing dormancy by many workers viz., Helgenson and Green, 1957; Corns, 1960; Wiberg and Kolk, 1960; Khare et. al., 1965 and Don, 1979.

The present findings that seed dormancy of grasses was reduced by various treatments, especially gibberellic acid is in conformity with many reports that germination is controlled by irrhibitors and promotors (Khan, 1971; Wareing et. al. 1972; Lewak, 1984; Kefeli, 1985 and Bewley and Black, 1985; Parihar, 1988).

This concept has attracted a great deal of attention. The concept attributes the control of dormancy to various growth regulators or hormone inhibitors, such as ABA, and promoters, such as gibberellins, cytokinins and ethylene. According to the theory, dormancy is maintained and possibly even induced by inhibitors and it can end only when the inhibitors are removed or when promotors overcome it. The theory owes its inception primarily to know effect of applied growth regulators on dormancy, some of which cause a dormant seed to germinate, whereas others inhibit germination of a non-dormant seed. A concept is that important metabolic changes occur as a consequence of the action of the dormancy breaking factor. One such change is thought to involve the synthesis of RNA and protein and others in the operation of the pentose phosphate path way (Copeland, 1976).

# 3. Effect of Storage (seed age) and Removal of Glumes on Germination:

Germinability of spikelets and seeds at three months intervals from the date of initial seed collection (seed obtained by dehusking of spikelets at three monthly intervals) were

studies. Results on viability in relation to age shoed that the percentage germination increased with increasing the storage period (Table 6a, b, Fig. 3A,B). The increasing trend in percentage germination was maintained up to 12 to 18 months of storage and thereafter, decline in germination percentage was observed. The spikelets as well as seeds showed primary dormancy for a period of about three months and thereafter, it seems that only secondary dormancy was in operation. Enhanced germination after a period of after ripening, may be attributed to the loss or decrease in the level of endogenous inhibitors as well as synthesis of germination promotors as discussed earlier.

A comparison of percentage germination between spikelets and seeds revealed that removal of glumes has an enhanced effect on percentage germination. Many workers viz., Toole,1939, 40, a,b, 41; Alkamine, 1944; Elliot and Leopold, 1953; Mattrnura and Hirayoshi, 1961; Ahring, 1963; Hagon, 1976; Tothill, 1977; Pandeya and Jayan, 1978; Juntilla, 1977; Whiteman and Mendra, 1982; Parihar et al. 1984 a,b have shown that seeds of various grasses germinate better when the glumes are removed. This enhanced germination of seeds in comparison to spikelets may be attributes to the removal of inhibitors (phenolics) present in the seed enclosing structure as well as easy availability of oxygen to embryo and also removal of mechanical restrain imposed by glumes over the seeds. Perusal of data (Table 6 b, Fig. 3B) also indicate that the effectiveness of this treatment (removal of glumes) is reduced in the latter stages of storage periods indicating that there is no secondary dormancy in operation at the latter stages.

The results obtained in present studies on storage of five range grasses conformity with the results reported by several workers (Pathak et. al., 1976; Parihar et. al. 1984 a,b; Parihar and Rai, 1985; Parihar, 1986; Parihar, 1988; Conde, et. al.,1995). However,

Mâtias and Bilbao (1985) reported in Cuba that in case of <u>P</u>. <u>maximum</u> cultivars viz., SIH 127 and local ecotype showed highest germination of 12.5 and 9.4%, respectively after two months of storage and after that the germination percentage dropped.

#### 4. Rate of Germination:

The germination of spikelets was uneven and extended for a larger period as compared to seeds (Table 7a,b, Fig. 4a,b). It may be possible that the germination of spikelets is delayed due to the retention of the endogenous inhibitors present in the caryopsis (seed) by exogenous inhibitors present in the seed enclosing glumes or by the reduction of oxygen transport to embryo as discussed earlier. All these factors may also have a cumulative effect resulting in the uneven germination of spikelets. Elimination of these inhibitory effect might have resulted even and early germination of seeds.

Germination curves (Fig. 4) are sigmoid- a minority of spikelets in the population germinate early, then the germination percentage increases more or less rapidly and finally the relatively few late germinations emerge. All the curves are positively skewed, because a greater percentage germination in the first half of the germination period—than in the second one. Though—the curves have the same general shape, but important behavior between populations are evident. For example, the germination curves of Chrysopogon fulvus flattens off when only a low percentage of spikelets have germinated, showing that this population has a low germination capacity i.e. the portion of spikelets capable of complete germination is low. The shape of curves also depended on the uniformity of the population, i.e. the degree of simultaneity or synchrony of germination.

Removal of glumes enabled an even and comparatively quick germination in seeds, as represented by the seed curves, which evidently confirmed the inhibitory effect due to glumes as well as due to exogenous phenolics present in the husk. Tothill (1977) also observed a very great increase in the rate of germination in caryopsis of Heteropogon contortus due to removal of hull and concluded that very uneven germination of spikelets is probably an adaptive characteristic, since the rains are also sporadic and uncertain and it would not be beneficial for all the seeds to germinate at one time.

Similarly, Parihar (1988) also observed an increase in the rate of germination in the seeds of <u>S. nervosum</u>, <u>C. fulvus</u>, <u>B. pertusa</u>, <u>B. intermedia</u> and <u>P. pedicellatum</u> due to removal of husk. He concluded the delayed germination of spikelets is due to retention of germination inhibitors (Exogenous as well as endogenous).

## 5. Phenolic Compounds in the Dispersal Units of Grasses and Implicated in Allelopathy:

The characterised phenolics isolated from the diaspore of five range grasses fall in the following three categories (Table20, Fig.10 and 11).

Table 20: Phenolic compounds encountered in the dispersal units of range grasses

No. of carbon atom	Basic skeleton	Class	Examples
7	C <sub>6</sub> - C <sub>1</sub>	Phenolic acids	p-hydroxy benzoic acid and vanillic acid
9	C <sub>6</sub> - C <sub>3</sub>	Hydrocinnamic acids	Caffeic acid, Ferulic acid and p- coumaric acid
15	C <sub>6</sub> - C <sub>3</sub> -C <sub>6</sub>	Flavonoids	Cyanidin

p-hydroxy benzoic acid

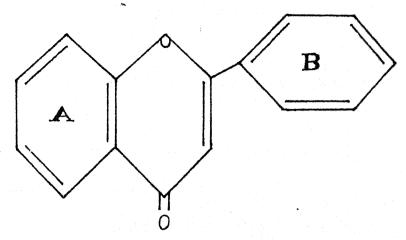
Caffeic acid

$$CH = CHCO_2H$$
  $HO$ 

CH=CHCO\_2H

P-coumaric acid

Fig.10: Phenolic and hydroxy cinnamic acids encountered in the dispersal units of range grasses.



- Flavone skeleton

Cyanidin

Fig.ll: Flavonoids (Aglycone) encountered in the dispersal units of range grasses.

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- The compounds having 7 carban atom with a basic skeleion of  $C_{\epsilon}$ - $C_{1}$ , classed under phenolic acids i.e. p-hydroxy benzoic acid and vanillic acid.
- The compunds having 9 carbon atom with a basic skeleton of  $C_6$ - $C_3$ , classed under hydrocinnamic acids i.e. Caffeic acid, Ferulic acid and p-coumaric acid.
- The compounds having 15 carban atom with a basic skeleton of  $C_6$ - $C_3$ - $C_6$ , classed under flavonoids i.e. Cyanidin.

In the present studies, isolation of phenolic acids in the diaspores (spikelets) of range grasses, revealed that p-hydroxy benzoic, Vanillic, Caffeic, Ferulic and p-coumaric acid were found in <u>C</u>. <u>fulvus</u> while incase of <u>P</u>. <u>pedicellatum</u> p-hydroxy benzoic, Caffeic, Ferulic and p-coumaric acid were observed. In case of <u>D</u>. <u>annulatum</u> p-hydroxy benzoic, Vanillic and p-coumaric acids were seen. In <u>B</u>. <u>intermedia</u> p-hydroxy benzoic, Vanillic, Ferulic and p-coumaric acids were isolated. While in <u>P</u>. <u>maximum</u> p-hydroxy benzoic, vanillic, Caffeic and Ferulic acids were observed.

According to Harborne (1980), four phenolic acids viz., p-hydroxy benzoic acid, protocatechuic acid, vanillic acid and Syringic acids are universally distributed in plants and the most common hydrocinnamic acid of plant is caffeic acid followed by ferulic and sinapic acids. Parihar (1988) reported the distribution of phenolic acid in the diaspores of range grasses, p-hydroxy benzoic, vanillic, p-coumaric and ferulic acids were found in B. pertusa, C. fulvus and S. nervosum. Protocatechuic acid was found in B. pertusa, syringic and caffeic acids were isolated from C. fulvus. The presence of phenolic and hydrocinnamic acids in the diaspores of B. intermedia and P. pedicellatum can not be ruled out.

Flavonoids forms the largest group and have C<sub>15</sub> skeleton of flavone (Fig. 11).

Among the anthocyanins, the crimson cyanidin occurs very widely and which gives the

purple tense to the spikelets of P. pedicellatum and B. intermedia (in the early flowering stage only). All these flavanoids have a similar hydroxylation in the A-ring and differ mainly in the oxidation level of the central pyron nucleus and in the number of hydroxy group in the B-ring one, two, or three.

The phenolic acids as well as hydroxy cinnamic acids have been the most commonly identified toxins produced by higher plants and involved in allelopathy. As back as 1908, Schreiner and Reed reported that vanallin, vanillic acid, and hydroxy quinone are among phenolic compounds produced by plants that are inhibitory to seed germination, and seedling growth.

p-hydroxey benzoic acid, vanillic acid and syringic acid are the most commonly identified phenolic acids involved in allelopathy. These are identified as important inhibitors in many cases e.g. in residue of corn, wheat, sorghum and oats (Guenzi and McCalla, 1966a) in cropped soil (Guenzi and McCalla, 1966 b), and in sugar beet flower clusters (Battle and Whittngton, 1969; Chandra Mohan et. al. 1973), isolated vanillic acid, p-hydroxy benzoic acid from the rice field soil at Annamalainagar (S. India). Chou and Young (1975) isolated p-hydroxybenzoic acid, vanillic acid and syringic acids as phytotoxins in 12 species of subtropical grasses.

The most commonly identified hydroxy cinnamic acids involved in allelopathy are p-coumaric acid, caffeic acid, ferulic acid, chlorogenic acid and sinapic acid.

Schreiner and Reed (1908) demonstrated that cinnamic acid, o-coumaric acid are among several organic compounds known to be produced by plants that are inhibitory to seedling growth. Chlorogenic and caffeic acids are fungistatic agents produced by potatoes in reponse to inoculation with <u>Helminthosproum carbonum</u> (Kuc et. al., 1956).

Several cinnamic acid derivatives were identified as germination inhibitors in many species (Van Sumere and Massart, 1959). Ferulic and p-coumaric acids are among the inhibitors present in the residue of corn, wheat, sorghum and oats and in soil under these crops (Guenzi and McCalla, 1966 a, b). Chlorogenic acid and p-coumaric acid are inhibitors in Sorghum halepense (Abdul-Wahab and Rice, 1967). Ferulic and p-coumaric acids are two of the inhibitors present in the flower clusters of sugar beet (Battle and Whittington, 1969). Caffeic, chlorogenic, p-coumaric and ferulic acids are among the toxins in the leaf leachate of Eucalyptus canaldulensis (del Moral and Muller, 1970). Ferulic and p-coumaric acids are common phytotoxin in 12 species of grasses (Chou and Young, 1975).

Ferulic acid has also been identified as an N-acyl terminal group in a protein of barley seed (Van Sumere et. al., 1972). Ferulic acid also occurs dimmersied as diferulic acid bound to carbohydrate of cell wall in a number of grasses (Hartley and Johns, 1976). Ferulic acid has also been found as a general germination inhibitors in cereals seeds (Van Sumere et. al., 1972). Ferulic, p-coumaric and caffeic acids has also been found as an acyl residue of cyanidin glycoside in the infloresence of grass (Harborne, 1967; Parihar and Patil, 1984).

Flavonoids forms the largest group and are wide spread in seed plants (Harborne and Simmonds, 1964). According to Rice (1984), "In spite of large number and wide distribution only a relatively small number have been reported as toxins implicated in allelopathy".

The anthocyanin colouration is frequently present in inflorescence, glumes, roots and seeds of grasses, though from their superficial appearance, the grasses would not

seem to be a good source of these pigments. Cyanidin glycosides seem to be characteristics anthocyanin of the grasses and cyanidin 3-glucoside has been found in nearly every grass examined by Harborne and his associates. Harborne, 1967; Parihar and Patil, 1984; Parihar, 1986b; Parihar and Kanodia 1986 and 87 found cyanidin 3 - arabinoside, acylated with cinnamic acid derivatives in the spikelets of <u>C. ciliaris</u>, <u>C. setegerus</u> and <u>Dichanthium annulatum</u>. Li, et. al. (1993) found trans-cinnamic acid, caffeic acid, ferulic acid, chlorogenic acid, abscisic acid (ABA), o-coumaric acid, m-coumaric acid, p-coumaric acid or coumarin in lettuce seed.

#### 6. Bioassay Studies:

Results of bioassay studies have revealed that water extract of diaspore had an inhibitory effect on percentage seed germination and root and shoot growth of the respective grass species as well as test species, though the extent of inhibition varied from species to species (Table 15a to 19c). A varieties of phenolic compounds involved in allelopathy and identified in the water extract of diaspores have already been discussed in the previous chapter. Therefore, the inhibitory effect on germination and root as well as shoot growth of water extract of diaspore was obviously due to the presence of these secondary compounds.

As regards to their mode of action, resulting in an inhibitory effect, Einhellig (1986) has stated "The elucidation of the methods where by allelopathic chemical alter plant growth and development has been a difficult and on going challenge. A clear insight into the precise physiological perturbations caused by these substances has not been obtained and it must be emphasized that much additional information is needed".

Some of the explicit references concerning certain mechanism of inhibition due to allelopathic compounds has been given by Horsley,1977; Rice, 1984; Balke, 1985; Einhellig et. al., 1985; Einhellig, 1986 and Parihar 1988. It may be concluded that allelochemicals interfere with many of the primary metabolic processes and growth regulatory system of higher plants.

However, before discussing the physiological alternations caused by allelochemicals, t should be recognized that their biological activity in concentration depend with a response threshold. Mild growth reduction above the threshold often show no-visible sign of injury and in some instances growth is stimulated below the threshold concentration. The inhibition threshold for specific substance is not a constant, but is intimately related to the sensitivity of receiving species and the environmental condition. For example, the threshold for reducing the growth of grain of sorghum (Sorghum bicolor (L) Monech) and seedling by several cinnamic acids was 1/25 that required to inhibit germination and initiation of ferulic acid inhibition of these seedlings under high temperature occurred at about one half the concentration required at moderate temperature (Enhellig, 1986).

Reduction in root growth by preventing or slowing the mitosis by various kinds of phenolic compounds has been reported (Cornman, 1946; and Avers and Goodwin, 1956). Several phenolic allelochemicals modify biosynthesis of major plant constituents or the distribution of carl: an in cellular pool. Cameron and Julian (1980) also observed inhibition of protein synthesis by cinnamic and ferulic acids in lettuce.

Allelochemicals also influence the delicate balance of hormones involved in regulating plant growth. One mechanism of allelopathic action for several phenolic

substances is ar alteration of the level of indole acetic acid (IAA). Either inhibition or stimulation of IAA has been reported by chlorogenic, cinnamic and benzoic acids (Tomaszewski and Thimann, 1966). Lee et. al. (1982) corroborated that the phenotic acid can be divided into two groups, suppressors of IAA destruction viz., chlorogenic, caffeic, ferulic and protocatechuic acids and those that stimulate IAA oxidase viz., p-coumaric, p-hydroxy benzoic, vanillic and syringic acids.

Inhibition of seed germination and root and shoot growth has also been explained due to the interference in respiratory metabolism. This is supported by the fact that water extract from allelopathic plant and residues may either decrease or increase the respiration of test plants (Patrick and Koch, 1958; Lodhi and Nickell, 1973).

The studies indicated that the greatest breath of secondary effect occurred in the inhibition of seed ing growth, where it was suspected to be the collective disruption of multiple plant functions that translate into disorganisation and impairment of growth. Perhaps this give some rational for the fact that seedling growth is typically more sensitive to allelochemicals interference than seed germination.

## 7. The Possible Ecological Significances of Phenolics Present in the Dispersal Units of Grasses.

Since the discovery of germination inhibitors in the early part of 20th century, it has been widely acknowledged that presence of germination inhibitors in the seeds, fruits, propagules etc. is an ecological adaption. The possible ecological importance of these germination inhibitors has been discussed by number of workers and currently it is one of the exciting area of research in the ecology. Some of the important contributions in

understanding the ecological role has been made by Went, 1948, 49, 57; Evenari, 1949, 57, 61; Kollar, 1955, 72; Kollar and Negbi, 1959; Oppenheimer, 1960; Audus, 1965; Wareing, 1965; Roberts, 1965; Barton, 1965a, b; Wareing and Saunders, 1971; Kefeli and Kadyrov, 1971; Thompson, 1973; Mayer and Shain, 1974; Mayer and Poljakoff - Mayber, 1982; Bewley and Black, 1985 and many others.

One of the most widely acclaimed role of germination inhibitors is that occurrence of these results in a sporadic germination over extended period of time (Evenari, 1949, 57; Wareing, 1965; Audus, 1965) where the dispersal units, whether seeds, fruits or aggregates of fruits contain inhibitors the latter are removed by rain and bacterial action at different rates thus causing irregular germination over an extended period. In case Sinapsis, for example the seeds contained in the beaks germination later than those released from the valve. In this way the risk of total experimentation of a crop of seedling because of adverse condition is likely to be avoided.

In some other cases these germination inhibitors act like a raingauge and determine the extent of rainfall (Weather) sufficient to support the seedling emerged from the seeds. As it has been observed that in desert habitats germination is rigorously linked to water supply if the newly emerged seedlings are going to survive. This means that seedling should not emerge when there is just enough water to support germination but rather when the soil has enough water to sustain subsequent plant growth. Some of the interesting findings by Went (1948, 49) on germination physiology of certain desert plants suggest how this height occur. Many species in desert soil germinate only after heavy rains (e.g. 12-15 mm), but not after light rain, even when the latter is composed of several, intermittent falls. Moreover, the precipitation (says 15 mm) is more effective when spread

over 10 hrs than when received over one hour. The explanation for this is that the seeds (e.g. of <u>Euphorbia</u> spp. and <u>Pectis papposa</u>) contain water soluble germination inhibitors, which must be leached out before germination can commence. Thus, the seeds have a device that acts like a raingauge and insures that germination takes place only when the earth has received sufficient rain to support the establishment and growth of the seedlings.

However, Mayer and Poljakoff - Mayber (1982) are of the opinion that these presumed functions of inhibitors in fruits are by no means finally proven, since it is possible to interpret the observed facts differently.

The chemical inhibitors are compounds termed as secondary substances, though Bell and Charlwood (1980) stated. "It is pity indeed that the term 'Secondary' should never have been applied to these compounds as a word gives the unfortunate impression that they are all relatively unimportant. By definition they do not play a role in the primary metabolic processes essential for maintenance of life in an individual plant, neverthless many may be absolutely essential for the maintenance of survival of the species as whole in a given natural habitat."

Since 1970 allelopathy attracted wide attention among scientists and there has been an exponential increase in the rate of publication. The surge of activity included several international meetings (Putnam and Tang, 1986 b), often followed by special journal issues (e.g. <u>Journal of chemical ecology</u> 9(8): 1983) and symposium series such as <u>Recent advances in Phytochemistry Vols</u>. 3,4,10,12,19 and 20.

As a result of these activities a large number of publication appeared especially since 1969-70 indicating a growing interest in the chemical and biochemical aspects of ecology and these included books, multiauthor volumes as well as shorter reviews as already mentione 1.

Therefore, Swain in 1979 wrote "over the past 10 years a subtle change has taken place in the attituce of chemists, ecologists and even taxonomists as to the importance of the so called secondary plant compounds. Previously, these substances were regarded as providing a challenge only to organic chemists interested in structural elucidation or biosynthetic speculation, but otherwise were thought of as waste product of plant metabolism which might be of limited taxonomic use. Now a majority of workers of these and cognate field believe that at least a few of these compounds are of direct importance in determining certain interactions between plants and other organisms in a given ecosystem".

While discussing the role of secondary compounds in the diaspores of grasses and before going further, it is perhaps right to ask whether there is any justification for supposing that these compounds have an ecological role at all. In practice, we know so little about most of the secondary compounds that a general answer to this question is not possible. There is however, no reason to suppose that the biochemistry of a plant is any less subject to the secondary pressures exercised by its environment than in the plants physiology or morphology, nor to suppose that the morphologically inefficient one. Bell (1980) suggested that during the course of evolution millions of secondary products have been synthesised from time to time by different species of plants and when the presence of a particular secondary product has conferred a selectionary advantage on the plant containing it, then the chances of survival of the plant, its offspring, and the secondary product itself will have been enhanced. The great majority of secondary product thrown up during the course of evolution probably proved to be of no advantage to the plants which synthesised them and neither the plants nor the compounds are likely to have survived for our study today. Such random synthesis of new compounds by plants have

lower, provided the variability which is prerequisite for the operation of natural selection and the advancement of all the living organisms.

Bell (1978) also suggested that plants synthesis a greater array of secondary compounds than animals because plants can not rely on physical mobility to escape their predators and have therefore, evolved a chemical defence against such predators.

Therefore, an attempt is made to ellucidate the possible role of phenolics present in the dispersal units of grasses in the context of chemical defence against microorganisms and herbivores. This defence mechanisms would be essential for the very survival of species during the course of evolution in a natural ecocystem.

However, defence against pathogens and herbivores may be secured by many different mechanisms viz., it may depend on the texture and composition of plant surface (Preece and Dickenon, 1971), the presence of anatomical structures such as thorns or resin ducts (Maxwell et. al., 1972), the absence of nutrients required by the pest (House, 1961), unsuitable pH or osmtic pressure (Painter, 1951) or accumulation of secondary products (Chapman, 1974; Fawcett and Spencer, 1969; Stoessl, 1983; Levine, 1976; Friend, 1979; Harborne, 1977a, 78, 80; Swain, 1977, 79; Bell, 1980, 81 and Janzen, 1983).

## I. Phenolics in Pathogen Resistance and Prevention of Seed Decay Before Germination:

Chemical resistance to attack have been classified as constitutive or induced (Levin, 1976). Constitutive resistance is based upon the presence of inhibitors prior to contact; most resistance to feeding by animals is constitutive. Induced resistance is based upon the accumulation and modification of normal host metabolites as a consequence of physical and chemical interactions between the plant and the attacker; most resistance to

bacteria, viruses, fungi and nematodes is induced.

The phenolics are the most important class of compounds in both constitutive and induced disease resistance (Levin, 1976; Friend, 1979). One of the best authenticated case of constitutive resistance involves the onion and the fungus <u>Colletotrichum circinans</u> (Walker and Stahmann, 1955). Some varieties of onion are resistant to fungus; the resistance is due to the presence of catechol and protocatechuic acid in the dead outer scales. These compounds are water soluble and diffuse from the dead cell layers of the scale to the infection drop, where they inhibit germination and penetration.

Fungistatic phenolic compound have been found in the protective layer of pea seeds. Schneider (1952) and Clauss (1961) reported that the seed coat of varieties resistant to causal organism of root disease contains higher level of phenolics than susceptible varieties. Hulme and Edney (1960) demonstrated that cyanidin in saturated aqueous solution completely inhibits the germination of <u>Glocosporium perenans</u>, the fungus responsible for apple fruit rot. Cyanidin occurs as the 3-galactoside in apple skins.

There are large number of instances where phenolics are important in both constitutive and induced disease resistance (Friend, 1979; Harborne, 1979; Swain, 1979). This evidence does not relate directly to the preventation of seed decay, but it certainly has important indirect relation. The compounds have to be inhibitory to bacteria and fungi to prevent disease caused by these organisms. Therefore, there appears to be no doubt that they inhibit growth of organisms involved in seed decay also (Swain, 1979, Rice, 1984). Rice (1984) believed that most seeds that do not germinate rapidly after landing in soil would be decomposed before germination if they did not contain microbial inhibitors, or in other words, phytoncides. According to Rice (1984) this may be one of the most consistent and important ecological role of allelopathy in annual and perennial plants growing in

natural areas, though the limited amount of research work available in this field does not reflect its importance and it appears that this is a fruitful phase of allelopathy for future research. It is very unlikely that seeds could remain in soil for a longer period without being decomposed if they did not contain microbial inhibitors. Such a mechanism may not be as critical in crop plants because they have been selected over a long period of time for rapid seed germ nation. Such seeds germinate rapidly so that they do not allow time for decomposition.

According to Tang and Zhang (1986), the importance of these inhibitors as preservatives require further research, while Kefeli and Dashek (1984) and Kefeli (1985) are of the opinion that these chemicals should not be considered as inhibitors.

#### II. Phenolics and Resistance to Phytophagous Insects:

Phenolic compounds in the plants are known to have both toxicological and behavioral effect on many groups of organisms including herbivorous insects, manimals, reptiles and mollusca (Wallace and Mansell, 1976). According to Feeny (1976) plant species or at least population may be regarded as chemically defended 'islands', subject to colonization by insects population and species in evolutionary time and the chemical defence of most r lant species are likely to be a consequence of co-evolution with a variety of predators, pair sites, pathogens and competitors.

According to Janzen (1983) "The physiological traits of seed are generated by sibling rivalry, need for protection during development and dispersal, parental resource allocation considerations, dispersal agents traits, and resource need by the young seedling". He further states "In addition to being hard a seedcoat may be very rich in secondary defence compounds".

There are many examples, where phenolics have been found to act as protective agents for seed plant and have both toxicological and behavioral effects on insect and the work has been reviewed by Detheir, 1970; Gilbert and Raven, 1975; Feeny, 1975; Wallace and Mansell, 1976; Bell, 1978; Swain, 1979; Edward and Wratten, 1980; Fox, 1981; Willson, 1983; Janzen, 1983 and many others.

For example, Shaver and Lukefar (1969) studied the effect of isoquercetin, morin, quercetin, rutin, quercetrin and hesperidin in synthetic diets on the growth and development of bollworm, tobacco budworm and pink bollworm. At concentration of 2% isoquercetin, quercetin and quercetrin caused a decrease in weight of bollworm pupae and an increase in the number of days required for pupation. All the flavonoides except morin reduced pupae weight and days required for pupation in tobacco budworm at concentration of 0.2%. Emergence of tobacco budworm pupae from larvae reared on diets with 0.4% quercetin, rutin and quercetrin was reduced by about 40% compared to control.

Caffeic acid, p-coumaric acid and ferulic acid presumably as their naturally occurring esters have been shown to affect the viability of or act as feeding deterrents to a variety of animals. Both p-coumaric and ferulic acids are totally lethal to cowpea weavil, Callosabruchus maculatus (Brucidal) when added at a concentration of 5% to a control diet, the latter acid showing significant effect at the 0.1% dose level(Janzen et. al., 1977). Chlorogenic acid reduces feeding of the diphagous Locusta migratoria by 50% when added at 2% to a standard wheat flour water (cited in Swain, 1979). A most interesting finding is that both p-coumaric and ferulic acids inhibit reproductive functions in the mountain vole (Microtus montanus) when fed at a rate of 4 mg/g daily for 12 days. Therefore, it is unlikely that those phenolics would act as germination inhibitors, though Janzen (1983) has also

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stated "Distinguishing between compounds functional as germination inhibitors and as predator deterrents is literally impossible at this stage of knowledge of seed natural history and in fact many compounds may regularly serve both functions".

In dicussing the role of toxic compounds in seeds, Bell (1981) has appropriately written "A seed cloes not exist in an ecological vacuum but in a complex living web which notudes the seed's parent plants and all other living species with which the plant and seed intract. This implies that the presence of a secondary compound in seed will be accompanied by other related adaptations (morphological, biochemical and physiological) in the seed in the whole plant and in the interacting species. These adaptations will result from the progressive modification derived from the presence of secondary compound and minimize any evolutionary disadvantage". This statement is true for the seeds of range grasses though the clear indication of the significance of a secondary compound may well be provided by the biochemical responses it elicits in other organisms.

#### III. Allelopathic Growth Stimulation:

According to Rice (1986) most research projects in allelopathy have been designed in such a way that only the inhibitory results are considered significant in explaining the biological problem under investigation. Thus any resulting stimulatory effects have been ignored or mentioned only incidentally. There are also reports about stimulation of seed germination (Rice, 1986). Therefore, possibly secondary substances in the diaspore may also have stimulatory effect. Lahiri and Kharbanda (1962) presumed that these germination inhibitors in various perennial fodder grasses are apt to counteract the natural propogation of vegetation in xeric environment and they also believed that these inhibitors caused important practical problem in the establishment of forage species after reseeding.

This seems to be an artifact of laboratory environment and any inhibitory effect that might, have on germination under field condition is either neutralized by soil or leached out by the rain or diluted to the extent that inhibitory effect is negated or possibly has stimulatory effects.

#### IV. Allelochemic Sphere of Germinating Seeds:

Tang and Zhang (1986) have studied the behaviour of germinating mung bean (Vigna radiata L.) and introduced the concept of allelochemic sphere. They have shown that when a seed of mung bean was planted in centre of petridish containing 1.5% agar, a light brown ring of exudates (Secondary compounds) inhibitory to lettuce (Lactuca sativa L.) seed germination and seedling growth was observed. Therefore, possibly the germinating dispersal units of grasses form an allelochemic sphere under field conditions and probably inhibit the germination and growth of other plant species in its close vicinity, thus giving an advantage of successful establishment to their own seedlings.

### V. Secondary compounds and grazing behaviour of animals

A large number of flavonoids derivatives (e.g. naringin) are among the most bitter substances known and have a role as feeding deterrent, protecting plants from over grazing (HarLorne, 1977a, 80; Swain, 1977, 79). Generally most of the grasses are unpalatable or less palatable to grazing animals at dead ripe stage. Therefore, possibly the accumulation of secondary substances in leaves also reflect the grazing behaviour of animals as the amount of cinnamic acid derivatives increases with the age in grasses (Harborne, 1977 a).

# SUMMARY

### SUMMARY

Studies on allelopathic potentials of five range grasses viz., <u>Bothriochloa intermedia</u> (Phulakara grass), <u>Chrysopogon fulvus</u> (Dhawlu grass), <u>Dichanthium annulatum</u> (Kail grass), <u>Panicum maximum</u> (Guinea grass) and <u>Pennisetum pedicellatum</u> (Deena Nath grass) were carried out from 1995 to 1997 with the following objectives.

- 1. To assess the seed dormancy, germinability and viability including effects of scarification/chemical treatments on dormancy and seed germinability.
- 2. To obtain information on effect of storage (seed age) and removal of glumes on germination so as to know the role of seed (caryopsis) enclosed by glumes etc. on seed germinability and viability.
- To isolate and characterize the germination inhibitors present in the dispersal unit by the combined use of chromatography, absorption spectroscopy and colour reactions.
- To conduct bioassay studies on the compounds extracted from the dispersal units and using these compounds on the respective grass seeds (caryopsis) and two test species.
- 5. To explain the possible ecological significance of these chemical compounds present in the dispersal units in the light of recent work carried out on this important field of all-lopathy.

The results obtained on various aspects of seed germination and viability with an emphasis on allelopathic potentials are summarised as under:

#### 1. Dormancy:

Studies on dormancy revealed that freshly collected spikelets of all the grasses did not germinate except <u>C</u>. <u>fulvus</u> (3.6%) and <u>D</u>. <u>annulatum</u> (3.6%). Further removal of husk (glume) from the seeds resulted in higher germination in <u>C</u>. <u>fulvus</u>, <u>D</u>. <u>annulatum</u> and <u>B</u>. <u>intermedia</u>. However, in case of <u>P</u>. <u>pedicellatum</u> and <u>P</u>. <u>maximum</u> dehusking could not facilitate germination even in freshly collected seed. The stored spikelets started breaking dormancy after storage of about three months in <u>P</u>. <u>pedicellatum</u> and <u>B</u>. <u>intermedia</u> while in case of <u>P</u>. <u>maximum</u> it required about six months. The inability of freshly harvested seed to germinate is termed as primary dormancy.

## 2. Effect of scarification and chemical treatments on germination of spikelets:

There was no beneficial effect of various scarification and chemical treatments for reducing the dormancy in freshly harvested spikelets, except <u>B</u>. <u>intermedia</u> where marginal dormancy was reduced with pre-chilling and heat treatment (2.7%). Gibberellic acid showed most effective in germination of freshly collected spikelets in case of <u>C</u>. <u>fulvus</u> and <u>D</u>. <u>annulatum</u>.

Enhancec germination of spikelets stored for nine months was observed with potassium nitrate in  $\underline{C}$ . <u>fulvus</u> and with hot water, heat and potassium nitrate in  $\underline{P}$ . <u>pedicellatum</u>. In  $\underline{D}$ . <u>annulatum</u> increase in germination was observed with pre-chilling, hot water, heat, potassium nitrate and gibberellic acid. However, there was no beneficial effect of scarification and chemical treatments for enhancing the germination in case of  $\underline{B}$ . <u>intermedia and  $\underline{P}$ . maximum</u>.

## 3. Effect of storage and removal of glumes on germination:

Results on viability in relation to storage periods revealed that percentage germination of spikelets increased with increasing the storage period up to nine months in all the grasses except <u>D</u>. <u>annulatum</u> where maximum germination (57.6%) was observed up to twelve months of storage. Thereafter, decline in germination percentage was observed. Thus the result showed that for higher establishment after collection of spikelets it should be shown next year.

Removal of husk (glume) showed enhanced effect on percentage germination in all the five range grasses which may be attributed to the removal of inhibitors present in the seeds. The maximum percentage germination was observed upto nine months in case of <u>C. fulvus</u> (84.8%), <u>P. pedicellatum</u> (85.6%), <u>B. intermedia</u> (78.4%) and <u>P. maximum</u> (69.6%) while in case of <u>D. annulatum</u> maximum germination (77.6%) was recorded in twelve months stored seeds.

#### 4. Rate of germination:

There was no appreciable difference in the rate of germination of spikelets and seeds in all the five grasses. However, higher germination percentage was recorded in seeds as compared to spikelets in all the species. Further it was observed that in case of C. fulvus, P. pedicellatum and B. intermedia takes shorter period to complete the germination as compared to D. annulatum and P. maximum.

## 5. Isolation and characterisation of isolation inhibitors:

Co-chromatographic examination of the chromatographically purified methanolic - HCl extract of the diaspore (spikelet) of  $\underline{P}$ . pedicellatum and  $\underline{B}$ . intermedia revealed the presence of cyanidin glycoside. In case of  $\underline{C}$ . fulvus, co-chromatography (with the authentic samples) of hydrolysed and chromatographically purified methanolic extract of

diaspores indicated presence of two phenolic acids viz., p-hydroxy benzoic and vanillic acids, three hydrocinnamic acids viz., caffeic, ferulic and p-coumaric acids. In P. pedicellatum p-hydroxy benzoic, caffeic, ferulic and p-coumaric acids were characterised. In case of D. annulatum p-hydroxy benzoic, vanillic and p-coumaric acids were seen. In B. intermedia p-hydroxy benzoic, vanillic, ferulic and p-coumaric acids were exhibited, while in P. maximum p-hydroxy benzoic, vanillic, caffeic and ferulic acids were observed.

#### 6. Bioassay studies:

Inhibition of seed germination as well as inhibition of root and shoot growth of five range grass species as well as two test species viz., Raphanus sativus and Vigna radiatus was recorded by the water extract of diaspore. However, the degree of inhibition varies depending upon the concentration of solution used for bioassay studies. The percentage inhibition also varied from species to species. However, in lower concentration less inhibition of germination or root and shoot growth was observed. Further, it was noted that there was no systematic trend in inhibition on germination, root and shoot length was seen in all the five grass species as well as two test species.

#### 7. The possible ecological significance of phenolics:

The phenolic compounds present in the dispersal units of grasses possibly plays important allelopathic roles viz., prevent the seeds from decaying and also act as a feeding deterrent for a variety of phytophagous insects in a natural ecosystem. There are a large number of examples where phenolics are important in both constitutive as well as induced disease resistance. This evidence does not relate directly to the preventation of seed decay, but it certainly has important indirect relation. The compounds have to be inhibitory to bacteria and fungi to prevent diseases caused by these organisms. Therefore, there appears to be no doubt that they inhibit growth of organisms involved in seed decay also.

Phenolic compounds in the plants/seeds are known to have both toxicological and behavioural effect on many group of organisms including herbivorous insects. For example, quercet n and its glycosides, p-coumaric, ferulic and caffeic acids etc. have been found to effect the viability of or act as feeding deterrent to a variety of phytophagous insects. Therefore, it is likely that phenolics in the dispersal units of grasses also protect the seed from predators in a natural ecosystem.

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# **APPENDICES**

### **APPENDICES**

Appendix-I

### Analysis of variance of test weight of range grasses

Source of variations	Degree of freedom		Mean sum of squ	lares
Variations	needon	Weight of spikelets(g)	Weight of seeds(g)	Weight of husks(g)
Replications	3	0	0.003	0.006
Treatments	4	4.75**	0.32**	3.180**
Error	12	0.01	0.01	0.014

<sup>\*\*</sup> Significant at 1% level.

Appendix-II

### Analysis of variance of scarification and chemical treatment on germination of spikelets at one month

Source of	Degree						
variations	of freedom	C. fulvus	C. fulvus P. pedicellatum a		B. intermedi a	P. maximum	
Replications	2	33.66	0	75.35	0	0	
Treatments	6	363.13**	0	489.24**	0	0	
Error	12	29.53	0	13.37	0	0	

<sup>\*\*</sup> Significant at 1% level.

Appendix-III

# Analysis of variance of scarification and chemical treatments on germination of spikelets at nine months

Source of	Degree of	Mean sum of squares					
variations	freedom	<u>C</u> . fulvus	P. pedicellatum	<u>D</u> . annulatum	B. intermedia	P. maximum	
Replications	2	28.44	1.58	11.83	14.94	11.91	
Treatments	6	867.24**	1635.69**	375.19**	1005.66**	162.14*	
Error	12	14.22	40.71	9.74	18.13	28.22	

<sup>\*</sup> Significant at 5% level, \*\* Significant at 1% level

# Analysis of variance of storage on germination

ce of tions         df         C. fulvus         P. pedicellatum         D. annulatum         B. inter           Spkts         Seed         Spkts         Seeds         Spkts         Spkts         Spkts           cations         4         16.68         28.35         39.80         6.99         20.77         19.86         47.50           ments         7         274.76**         456.85**         1032.02**         1205.71**         472.40**         461.34**         1389.64**           28         9.29         19.84         36.52         12.68         19.04         20.45         23.34							Mean sum	Mean sum of squares				•
Apkts         Seeds         Spkts         Seeds         Spkts         47.50         47.50         47.50           7         274.76**         456.85**         1032.02**         1205.71**         472.40**         461.34**         1389.64**           28         9.29         19.84         36.52         12.68         19.04         20.45         23.34	Source of variations	d.	C. fu	Ivus	P. pedic	ellatum	D. ann	ulatum	B. inter	media	P. maximum	imum
4         16.68         28.35         39.80         6.99         20.77         19.86         47.50           7         274.76**         456.85**         1032.02**         1205.71**         472.40**         461.34**         1389.64**           28         9.29         19.84         36.52         12.68         19.04         20.45         23.34			Spkts	Seed	Spkts	Seeds	Spkts	Seeds	Spkts	Seeds	Spkts	Seeds
7         274.76**         456.85**         1032.02**         1205.71**         472.40**         461.34**         1389.64**           28         9.29         19.84         36.52         12.68         19.04         20.45         23.34	Replications	4	16.68	28.35	39.80	6:33	20.77	19.86	47.50	17.02	4.00	16.23
28 9.29 19.84 36.52 12.68 19.04 20.45 23.34	Treatments	7	274.76**		1032.02**	1205.71**	472.40**	461.34**	1389.64**	259.12**	1368.59**	1593.85**
	Error	28	9.29	19.84	36.52	12.68	19.04	20.45	23.34	12.86	24.10	17.66

Spkts- Spikelets \*\* Significant at 1% level df= Degree of freedom

### Appendix -Va

# Analysis of variance of leachate of <u>C</u>. <u>fulvus</u> on germination, root and shoot length of <u>R</u>. <u>sativus</u>

Source of	Degree of	Mean sum of squares		
variations	freedom	Germination	Root length	Shoot length
Replications	4	14.18	9.42	76.12
Treatments	3	96.52**	3611.51**	69.40
Error	12	3.86	84.72	66.85

<sup>\*\*</sup> Significant at 1% level

### Appendix -Vb

Analysis of variance of leachate of C. fulvus on germination, root and shoot length of V. radiatus

Source of	Degree of		Mean sum of squares		
variations	freedom	Germination	Root length	Shoot length	
Replications	4	23.65	2.92	67.55	
Treatments	3	48.54**	373.0**	32.60	
Error	12	5.97	3.12	22.10	

<sup>\*\*</sup> Significant at 1% level

### Appendix-Vc

Analysis of variance of leachate of <u>C</u>. <u>fulvus</u> on germination, root and shoot length of <u>C</u>. fulvus

Source of	Degree of	T:	ean sum of squa	res
variations	freedom	Germination	Root length	Shoot length
Replications	4	9.25	16.60	80.20
Treatments	3	228.10**	565.46**	1361.40**
Error	12	34.82	12.17	43.86

<sup>\*\*</sup> Significant at 1% level

# Analysis of variance of leachate of $\underline{P}$ . $\underline{pedicellatum}$ on germination , root and shoot length of $\underline{R}$ . $\underline{sativus}$

Source of variations	Degree of			
	freedom	Germination	Root length	Shoot length
Replications	4	3.07	55.70	33.20
Treatments	3	37.51	3912.85	1318.0**
Error	12	12.10	2010.54	68.20

<sup>\*\*</sup> Significant at 1% level

### Appendix-VIb

# Analysis of variance of leachate of <u>P</u>. <u>pedicellatum</u> on germination, root and shoot length of <u>V</u>. <u>radiatus</u>

Source of variations	Degree of		Mean sum of squares		
	freedom	Germination	Root length	Shoot length	
Replications	4	27.15	5.85	20.45	
Treatments	3	367.46**	13.65	70.53	
Error	12	26.91	8.02	57.45	

<sup>\*\*</sup> Significant at 1% level

### Appendix-VIc

# Analysis of variance of leachate of <u>P</u>. <u>pedicellatum</u> on germination, root and shoot length of <u>P</u>. <u>pedicellatum</u>

Source of	Degree of		Mean sum of square	es .
variations	freedom	Germination	Root length	Shoot length
Replications	4	13.05	5.57	105.57
Treatments	3	325.66	73.80	166.12
Error	12	99.53	38.50	642.84

# Analysis of variance of leachate of <u>D</u>. <u>annulatum</u> on germination, root and shoot length of <u>R</u>. <u>sativus</u>

Source of variations	Degree of freedom		res	
		Germination	Root length	Shoot length
Replications	4	31.18	203.80	60.50
Treatments	3	48.90*	1992.0**	697.00*
Error	. 12	9.36	76.02	148.80

<sup>\*</sup> Significant at 5% level

### Appendix -VIIb

# Analysis of variance of leachate of <u>D</u>. <u>annulatum</u> on germination, root and shoot length of <u>V</u>. <u>radiatus</u>

Source of	Degree of		Mean sum of squares		
variations	freedom	Germination	Root length	Shoot length	
Replications	4	10.26	11.55	26.45	
Treatments	3	112.27**	120.18**	39.06	
Error	12	10.00	5.51	57.15	

<sup>\*\*</sup> Significant at 1% level

### Appendix-VIIc

# Analysis of variance of leachate of <u>D</u>. <u>annulatum</u> on germination, root and shoot length of <u>D</u>. <u>annulatum</u>

Source of variations	Degree of	Mean sum of squares		
	freedom	Germination	Root length	Shoot length
Replications	4	23.24	7.55	6.32
Treatments	3	17.54	531.33**	275.40**
Error	12	20.31	20.58	19.85

<sup>\*\*</sup> Significant at 1% level

<sup>\*\*</sup> Significant at 1% level

### Appendix- VIIIa

# Analysis of variance of leachate of <u>B</u>. <u>intermedia</u> on germination, root and shoot length of <u>R</u>. <u>sativus</u>

Source of variations	Degree of	·	Mean sum of squares		
	freedom	Germination	Root length	Shoot length	
Replications	4	17.76	159.42	117.80	
Treatments	3	210.92**	1404.60**	696.58**	
Error	12	10.13	127.39	32.16	

<sup>\*\*</sup> Significant at 1% level

### Appendix- VIIIb

### Analysis of variance of leachate of <u>B</u>. <u>intermedia</u> on germination, root and shoot length of <u>V</u>. <u>radiatus</u>

Source of variations	Degree of	Mean sum of squares		
	freedom	Germination	Root length Shoot length	
Replications	4	25.43	5.57	56.01
Treatments	3	159.00	117.64**	138.38*
Error	12	352.95	6.11	27.19

<sup>\*</sup> Significant at 5% level

### Appendix- VIIIc

# Analysis of variance of leachate of <u>B</u>. <u>intermedia</u> on germination, root and shoot length of <u>B</u>. <u>intermedia</u>

Source of	Degree of	Mean sum of squares		es
variations	freedom	Germination	Root length	Shoot length
Replications	4	5.35	96.75	9.55
Treatments	3	24.82	426.73**	41.46
Error	12	15.74	5.10	21.71

<sup>\*\*</sup> Significant at 1% level

<sup>\*\*</sup>Significant at 1% level

# Analysis of variance of $\underline{P}$ . $\underline{maximum}$ on germination, root and shoot length of $\underline{R}$ . $\underline{sativus}$

Source of variations	Degree of	Mean sum of squares		
	freedom	Germination	Root length	Shoot length 30.17
Replications	4	27.76	247.07	30.17
Treatments	3	1330.87**	9055.78**	1465.66**
Error	12	25.50	85.74	77.04

<sup>\*\*</sup> Significant at 1% level

# Analysis of variance of <u>P</u>. maximum on germination, root and shoot length of <u>V</u>. radiatus

Source of variations	Degree of	Mean sum of squares		
	freedom	Germination	Root length	Shoot length  24.7  161.93*
Replications	4	7.51	9.07	24.7
Treatments	3	94.29**	159.11**	161.93*
Error	12	9.75	6.74	33.26

<sup>\*</sup> Significant at 5% level

# Analysis of variance of P. maximum on germination, root and shoot length of P. maximum

Source of variations	Degree of	Mean sum of squares		
	freedom	Germination	Shoot length	
Replications	4	6.92	49.25	17.42
Treatments	3	11.35	112.58**	5.25
Error	12	79.71	5.12	64.45

<sup>\*\*</sup> Significant at 1% level

<sup>\*\*</sup> Significant at 1% level